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(57) Abstract: A method to prepare synthetic nucleic acid molecules having reduced inappropriate or unintended transcriptional characteristics when expressed in a particular host cell.



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## SYNTHETIC NUCLEIC ACID MOLECULE COMPOSITIONS AND METHODS OF PREPARATION

### Statement of Government Rights

5           The invention was made at least in part with a grant from the Government of the United States of America (grant DMI-9402762 from the National Science Foundation). The Government has certain rights to the invention.

### Background of the Invention

10           Transcription, the synthesis of an RNA molecule from a sequence of DNA is the first step in gene expression. Sequences which regulate DNA transcription include promoter sequences, polyadenylation signals, transcription factor binding sites and enhancer elements. A promoter is a DNA sequence  
15           capable of specific initiation of transcription and consists of three general regions. The core promoter is the sequence where the RNA polymerase and its cofactors bind to the DNA. Immediately upstream of the core promoter is the proximal promoter which contains several transcription factor binding sites that are responsible for the assembly of an activation complex that in turn recruits the  
20           polymerase complex. The distal promoter, located further upstream of the proximal promoter also contains transcription factor binding sites. Transcription termination and polyadenylation, like transcription initiation, are site specific and encoded by defined sequences. Enhancers are regulatory regions, containing multiple transcription factor binding sites, that can significantly increase the  
25           level of transcription from a responsive promoter regardless of the enhancer's orientation and distance with respect to the promoter as long as the enhancer and promoter are located within the same DNA molecule. The amount of transcript produced from a gene may also be regulated by a post-transcriptional mechanism, the most important being RNA splicing that removes intervening  
30           sequences (introns) from a primary transcript between splice donor and splice acceptor sequences.

Natural selection is the hypothesis that genotype-environment interactions occurring at the phenotypic level lead to differential reproductive success of individuals and therefore to modification of the gene pool of a population.

5 Some properties of nucleic acid molecules that are acted upon by natural selection include codon usage frequency, RNA secondary structure, the efficiency of intron splicing, and interactions with transcription factors or other nucleic acid binding proteins. Because of the degenerate nature of the genetic code, these properties can be optimized by natural selection without altering the  
10 corresponding amino acid sequence.

Under some conditions, it is useful to synthetically alter the natural nucleotide sequence encoding a polypeptide to better adapt the polypeptide for alternative applications. A common example is to alter the codon usage frequency of a gene when it is expressed in a foreign host cell. Although  
15 redundancy in the genetic code allows amino acids to be encoded by multiple codons, different organisms favor some codons over others. It has been found that the efficiency of protein translation in a non-native host cell can be substantially increased by adjusting the codon usage frequency but maintaining the same gene product (U.S. Patent Nos. 5,096,825, 5,670,356, and 5,874,304).

20 However, altering codon usage may, in turn, result in the unintentional introduction into a synthetic nucleic acid molecule of inappropriate transcription regulatory sequences. This may adversely effect transcription, resulting in anomalous expression of the synthetic DNA. Anomalous expression is defined as departure from normal or expected levels of expression. For example,  
25 transcription factor binding sites located downstream from a promoter have been demonstrated to effect promoter activity (Michael et al., 1990; Lamb et al., 1998; Johnson et al., 1998; Jones et al., 1997). Additionally, it is not uncommon for an enhancer element to exert activity and result in elevated levels of DNA transcription in the absence of a promoter sequence or for the presence of  
30 transcription regulatory sequences to increase the basal levels of gene expression in the absence of a promoter sequence.

Thus, what is needed is a method for making synthetic nucleic acid molecules with altered codon usage without also introducing inappropriate or unintended transcription regulatory sequences for expression in a particular host cell.

5

### **Summary of the Invention**

The invention provides a synthetic nucleic acid molecule comprising at least 300 nucleotides of a coding region for a polypeptide, having a codon composition differing at more than 25% of the codons from a wild type nucleic acid sequence encoding a polypeptide, and having at least 3-fold fewer, preferably at least 5-fold fewer, transcription regulatory sequences than would result if the differing codons were randomly selected. Preferably, the synthetic nucleic acid molecule encodes a polypeptide that has an amino acid sequence that is at least 85%, preferably 90%, and most preferably 95% or 99% identical to the amino acid sequence of the naturally-occurring (native or wild type) polypeptide (protein) from which it is derived. Thus, it is recognized that some specific amino acid changes may also be desirable to alter a particular phenotypic characteristic of the polypeptide encoded by the synthetic nucleic acid molecule. Preferably, the amino acid sequence identity is over at least 100 contiguous amino acid residues. In one embodiment of the invention, the codons in the synthetic nucleic acid molecule that differ preferably encode the same amino acids as the corresponding codons in the wild type nucleic acid sequence.

The transcription regulatory sequences which are reduced in the synthetic nucleic acid molecule include, but are not limited to, any combination of transcription factor binding sequences, intron splice sites, poly(A) addition sites, enhancer sequences and promoter sequences. Transcription regulatory sequences are well known in the art.

It is preferred that the synthetic nucleic acid molecule of the invention has a codon composition that differs from that of the wild type nucleic acid sequence at more than 30%, 35%, 40% or more than 45%, e.g., 50%, 55%, 60% or more of the codons. Preferred codons for use in the invention are those which are employed more frequently than at least one other codon for the same amino



acid in a particular organism and, more preferably, are also not low-usage codons in that organism and are not low-usage codons in the organism used to clone or screen for the expression of the synthetic nucleic acid molecule (for example, *E. coli*). Moreover, preferred codons for certain amino acids (i.e., those amino acids that have three or more codons,), may include two or more codons that are employed more frequently than the other (non-preferred) codon(s). The presence of codons in the synthetic nucleic acid molecule that are employed more frequently in one organism than in another organism results in a synthetic nucleic acid molecule which, when introduced into the cells of the organism that employs those codons more frequently, is expressed in those cells at a level that is greater than the expression of the wild type or parent nucleic acid sequence in those cells. For example, the synthetic nucleic acid molecule of the invention is expressed at a level that is at least about 110%, e.g., 150%, 200%, 500% or more (1000%, 5000%, or 10000%) of that of the wild type nucleic acid sequence in a cell or cell extract under identical conditions (such as cell culture conditions, vector backbone, and the like).

In one embodiment of the invention, the codons that are different are those employed more frequently in a mammal, while in another embodiment the codons that are different are those employed more frequently in a plant. A particular type of mammal, e.g., human, may have a different set of preferred codons than another type of mammal. Likewise, a particular type of plant may have a different set of preferred codons than another type of plant. In one embodiment of the invention, the majority of the codons which differ are ones that are preferred codons in a desired host cell. Preferred codons for mammals (e.g., humans) and plants are known to the art (e.g., Wada et al., 1990). For example, preferred human codons include, but are not limited to, CGC (Arg), CTG (Leu), TCT (Ser), AGC (Ser), ACC (Thr), CCA (Pro), CCT (Pro), GCC (Ala), GGC (Gly), GTG (Val), ATC (Ile), ATT (Ile), AAG (Lys), AAC (Asn), CAG (Gln), CAC (His), GAG (Glu), GAC (Asp), TAC (Tyr), TGC (Cys) and TTC (Phe) (Wada et al., 1990). Thus, preferred "humanized" synthetic nucleic acid molecules of the invention have a codon composition which differs from a wild type nucleic acid sequence by having an increased number of the preferred

human codons, e.g. CGC, CTG, TCT, AGC, ACC, CCA, CCT, GCC, GGC, GTG, ATC, ATT, AAG, AAC, CAG, CAC, GAG, GAC, TAC, TGC, TTC, or any combination thereof. For example, the synthetic nucleic acid molecule of the invention may have an increased number of CTG or TTG leucine-encoding codons, GTG or GTC valine-encoding codons, GGC or GGT glycine-encoding codons, ATC or ATT isoleucine-encoding codons, CCA or CCT proline-encoding codons, CGC or CGT arginine-encoding codons, AGC or TCT serine-encoding codons, ACC or ACT threonine-encoding codon, GCC or GCT alanine-encoding codons, or any combination thereof, relative to the wild type nucleic acid sequence. Similarly, synthetic nucleic acid molecules having an increased number of codons that are employed more frequently in plants, have a codon composition which differs from a wild type or parent nucleic acid sequence by having an increased number of the plant codons including, but not limited to, CGC (Arg), CTT (Leu), TCT (Ser), TCC (Ser), ACC (Thr), CCA (Pro), CCT (Pro), GCT (Ser), GGA (Gly), GTG (Val), ATC (Ile), ATT (Ile), AAG (Lys), AAC (Asn), CAA (Gln), CAC (His), GAG (Glu), GAC (Asp), TAC (Tyr), TGC (Cys), TTC (Phe), or any combination thereof (Murray et al., 1989). Preferred codons may differ for different types of plants (Wada et al., 1990).

The choice of codon may be influenced by many factors such as, for example, the desire to have an increased number of nucleotide substitutions or decreased number of transcription regulatory sequences. Under some circumstances (e.g. to permit removal of a transcription factor binding site) it may be desirable to replace a non-preferred codon with a codon other than a preferred codon or a codon other than the most preferred codon. Under other circumstances, for example, to prepare codon distinct versions of a synthetic nucleic acid molecule, preferred codon pairs are selected based upon the largest number of mismatched bases, as well as the criteria described above.

The presence of codons in the synthetic nucleic acid molecule that are employed more frequently in one organism than in another organism, results in a synthetic nucleic acid molecule which, when introduced into a cell of the organism that employs those codons, is expressed in that cell at a level which is

greater than the level of expression of the wild type or parent nucleic acid sequence.

A synthetic nucleic acid molecule of the invention may encode a selectable marker protein or a reporter molecule. However, the invention applies to any gene and is not limited to synthetic reporter genes or synthetic selectable marker genes. In one embodiment of a synthetic nucleic acid molecule of the invention that is a reporter molecule, the synthetic nucleic acid molecule encodes a luciferase having a codon composition different than that of a wild type or parent *Renilla* luciferase or a beetle luciferase nucleic acid sequence. A synthetic click beetle luciferase nucleic acid molecule of the invention may optionally encode the amino acid valine at position 224 (i.e., it emits green light), or may optionally encode the amino acid histidine at position 224, histidine at position 247, isoleucine at position 346, glutamine at position 348 or combination thereof (i.e., it emits red light). Preferred synthetic luciferase nucleic acid molecules that are related to a wild type *Renilla* luciferase nucleic acid sequence include, but are not limited to, SEQ ID NO:21 (Rlucver2) or SEQ ID NO:22 (Rluc-final). Preferred synthetic luciferase nucleic acid molecules that are related to click beetle luciferase nucleic acid sequences include, but are not limited to, SEQ ID NO:7 (GRver5), SEQ ID NO:8 (GR6), SEQ ID NO:9 (GRver5.1), SEQ ID NO:14 (RDver5), SEQ ID NO:15 (RD7), SEQ ID NO:16 (RDver5.1), SEQ ID NO:17 (RDver5.2) or SEQ ID NO:18 (RD156-1H9).

The invention also provides an expression cassette. The expression cassette of the invention comprises a synthetic nucleic acid molecule of the invention operatively linked to a promoter that is functional in a cell. Preferred promoters are those functional in mammalian cells and those functional in plant cells. Optionally, the expression cassette may include other sequences, e.g., restriction enzyme recognition sequences and a Kozak sequence, and be a part of a larger polynucleotide molecule such as a plasmid, cosmid, artificial chromosome or vector, e.g., a viral vector.

Also provided is a host cell comprising the synthetic nucleic acid molecule of the invention, an isolated polypeptide (e.g., a fusion polypeptide

encoded by the synthetic nucleic acid molecule of the invention), and compositions and kits comprising the synthetic nucleic acid molecule of the invention or the polypeptide encoded thereby in suitable container means and, optionally, instruction means. Preferred isolated polypeptides include, but are not limited to, those comprising SEQ ID NO:31 (GRver5.1), SEQ ID NO:226 (Rluc-final), or SEQ ID NO:223 (RD156-1H9).

The invention also provides a method to prepare a synthetic nucleic acid molecule of the invention by genetically altering a parent (either a wild type or another synthetic) nucleic acid sequence. The method may be used to prepare a synthetic nucleic acid molecule encoding a polypeptide comprising at least 100 amino acids. One embodiment of the invention is directed to the preparation of synthetic genes encoding reporter or selectable marker proteins. The method of the invention may be employed to alter the codon usage frequency and decrease the number of transcription regulatory sequences in any open reading frame or to decrease the number of transcription regulatory sites in a vector backbone. Preferably, the codon usage frequency in the synthetic nucleic acid molecule is altered to reflect that of the host organism desired for expression of that nucleic acid molecule while also decreasing the number of potential transcription regulatory sequences relative to the parent nucleic acid molecule.

Thus, the invention provides a method to prepare a synthetic nucleic acid molecule comprising an open reading frame. The method comprises altering (e.g., decreasing or eliminating) a plurality of transcription regulatory sequences in a parent (wild type or a synthetic) nucleic acid sequence that encodes a polypeptide having at least 100 amino acids to yield a synthetic nucleic acid molecule which has a decreased number of transcription regulatory sequences and which preferably encodes the same amino acids as the parent nucleic acid molecule. The transcription regulatory sequences are selected from the group consisting of transcription factor binding sequences, intron splice sites, poly(A) addition sites, enhancer sequences and promoter sequences, and the resulting synthetic nucleic acid molecule has at least 3-fold fewer, preferably 5-fold fewer, transcription regulatory sequences relative to the parent nucleic acid sequence. The method also comprises altering greater than 25% of the codons in the

synthetic nucleic acid sequence which has a decreased number of transcription regulatory sequences to yield a further synthetic nucleic acid molecule, wherein the codons that are altered encode the same amino acids as those in the corresponding position in the synthetic nucleic acid molecule which has a decreased number of transcription regulatory sequences and/or in the parent nucleic acid sequence. Preferably, the codons which are altered do not result in an increase in transcriptional regulatory sequences. Preferably, the further synthetic nucleic acid molecule encodes a polypeptide that has at least 85%, preferably 90%, and most preferably 95% or 99% contiguous amino acid sequence identity to the amino acid sequence of the polypeptide encoded by the parent nucleic acid sequence.

Alternatively, the method comprises altering greater than 25% of the codons in a parent nucleic acid sequence which encodes a polypeptide having at least 100 amino acids to yield a codon-altered synthetic nucleic acid molecule, wherein the codons that are altered encode the same amino acids as those present in the corresponding positions in the parent nucleic acid sequence. Then, a plurality of transcription regulatory sequences in the codon-altered synthetic nucleic acid molecule are altered to yield a further synthetic nucleic acid molecule. Preferably, the codons which are altered do not result in an increase in transcriptional regulatory sequences. Also, preferably, the further synthetic nucleic acid molecule encodes a polypeptide that has at least 85%, preferably 90%, and most preferably 95% or 99% contiguous amino acid sequence identity to the amino acid sequence of the polypeptide encoded by the parent nucleic acid sequence. Also provided is a synthetic (including a further synthetic) nucleic acid molecule prepared by the methods of the invention.

As described hereinbelow, the methods of the invention were employed with click beetle luciferase and *Renilla* luciferase nucleic acid sequences. While both of these nucleic acid molecules encode luciferase proteins, they are from entirely different families and are widely separated evolutionarily. These proteins have unrelated amino acid sequences, protein structures, and they utilize dissimilar chemical substrates. The fact that they share the name "luciferase" should not be interpreted to mean that they are from the same family, or even

largely similar families. The methods produced synthetic luciferase nucleic acid molecules which exhibited significantly enhanced levels of mammalian expression without negatively effecting other desirable physical or biochemical properties (including protein half-life) and which were also largely devoid of known transcription regulatory elements.

The invention also provides at least two synthetic nucleic acid molecules that encode highly related polypeptides, but which synthetic nucleic acid molecules have an increased number of nucleotide differences relative to each other. These differences decrease the recombination frequency between the two synthetic nucleic acid molecules when those molecules are both present in a cell (i.e., they are "codon distinct" versions of a synthetic nucleic acid molecule). Thus, the invention provides a method for preparing at least two synthetic nucleic acid molecules that are codon distinct versions of a parent nucleic acid sequence that encodes a polypeptide. The method comprises altering a parent nucleic acid sequence to yield a first synthetic nucleic acid molecule having an increased number of a first plurality of codons that are employed more frequently in a selected host cell relative to the number of those codons present in the parent nucleic acid sequence. Optionally, the first synthetic nucleic acid molecule also has a decreased number of transcription regulatory sequences relative to the parent nucleic acid sequence. The parent nucleic acid sequence is also altered to yield a second synthetic nucleic acid molecule having an increased number of a second plurality of codons that are employed more frequently in the host cell relative to the number of those codons in the parent nucleic acid sequence, wherein the first plurality of codons is different than the second plurality of codons, and wherein the first and the second synthetic nucleic acid molecules preferably encode the same polypeptide. Optionally, the second synthetic nucleic acid molecule has a decreased number of transcription regulatory sequences relative to the parent nucleic acid sequence. Either or both synthetic molecules can then be further modified.

Clearly, the present invention has applications with many genes and across many fields of science including, but not limited to, life science research,

agrigenetics, genetic therapy, developmental science and pharmaceutical development.

### Brief Description of the Figures

5           Figure 1. Codons and their corresponding amino acids.

Figure 2. A nucleotide sequence comparison of a yellow-green (YG) click beetle luciferase nucleic acid sequence (YG #81-6G01; SEQ ID NO:2) and various synthetic green (GR) click beetle luciferase nucleic acid sequences (GRver1, SEQ ID NO:3; GRver2, SEQ ID NO:4; GRver3, SEQ ID NO:5; 10 GRver4, SEQ ID NO:6; GRver5, SEQ ID NO:7; GR6, SEQ ID NO:8; GRver5.1, SEQ ID NO:9) and various red (RD) click beetle luciferase nucleic acid sequences (RDver1, SEQ ID NO:10; RDver2, SEQ ID NO:11; RDver3, SEQ ID NO:12; RDver4, SEQ ID NO:13; RDver5, SEQ ID NO:14; RD7, SEQ ID NO:15; RDver5.1, SEQ ID NO:16; RDver5.2, SEQ ID NO:17; RD156-1H9, 15 SEQ ID NO:18). The nucleotides enclosed in boxes are nucleotides that differ from the nucleotide present at the homologous position in SEQ ID NO:2.

Figure 3. An amino acid sequence comparison of a YG click beetle luciferase amino acid sequence (YG#81-6G01, SEQ ID NO:24) and various synthetic GR click beetle luciferase amino acid sequences (GRver1, SEQ ID 20 NO:25; GRver2, SEQ ID NO:26; GRver3, SEQ ID NO:27; GRver4, SEQ ID NO:28; GRver5, SEQ ID NO:29; GR6, SEQ ID NO:30; GRver5.1, SEQ ID NO:31) and various red (RD) click beetle luciferase amino acid sequences (RDver1, SEQ ID NO:32; RDver2, SEQ ID NO:33; RDver3, SEQ ID NO:34; RDver4, SEQ ID NO:218; RDver5, SEQ ID NO:219; RD7, SEQ ID NO:220; 25 RDver5.1, SEQ ID NO:221; RDver5.2, SEQ ID NO:222; RD156-1H9, SEQ ID NO:223). All amino acid sequences are inferred from the corresponding nucleotide sequence. The amino acids enclosed in boxes are amino acids that differ from the amino acid present at the homologous position in SEQ ID NO:24.

Figure 4. Codon usage in YG#81-6G01, GRver1, RDver1, GRver5, and 30 RDver5, and humans (HUM) and relative codon usage in YG#81-6G01, GRver5, RDver5, and humans.

Figure 5. Codon usage summaries for YG#81-6G01 (Figure 5A), and GR/RD synthetic nucleic acid sequences, GRver1 (Figure 5B), RDver1 (Figure 5C), GRver2 (Figure 5D), RDver2 (Figure 5E), GRver3 (Figure 5F), RDver3 (Figure 5G), GRver4 (Figure 5H), RDver4 (Figure 5I), GRver5 (Figure 5J), RDver5 (5K).

Figure 6. Oligonucleotides employed to prepare synthetic GR/RD luciferase genes (SEQ ID Nos. 35-245).

Figure 7. A nucleotide sequence comparison of a wild type *Renilla reniformis* luciferase nucleic acid sequence Genbank Accession No. M63501 (RELLUC, SEQ ID NO:19) and various synthetic *Renilla* luciferase nucleic acid sequences (Rlucver1, SEQ ID NO:20; Rlucver2, SEQ ID NO:21; Rluc-final, SEQ ID NO:22). The nucleotides enclosed in boxes are nucleotides that differ from the nucleotide present at the homologous position in SEQ ID NO:19.

Figure 8. An amino acid sequence comparison of a wild type *Renilla reniformis* luciferase amino acid sequence (RELLUC, SEQ ID NO:224) and various synthetic *Renilla reniformis* luciferase amino acid sequences (Rlucver1, SEQ ID NO:225; Rlucver2, SEQ ID NO:226; Rluc-final, SEQ ID NO:227). All amino acid sequences are inferred from the corresponding nucleotide sequence. The amino acids enclosed in boxes are amino acids that differ from the amino acid present at the homologous position in SEQ ID NO:224.

Figure 9. Codon usage in wild-type (A) versus synthetic (B) *Renilla* luciferase genes. For codon usage in selected organisms, see, e.g., Wada et al., 1990; Sharp et al., 1988; Aota et al., 1988; and Sharp et al., 1987, and for plant codons, Murray et al. 1989.

Figure 10. Oligonucleotides employed to prepare synthetic *Renilla* luciferase gene (SEQ ID Nos. 246-292).

Figure 11. A nucleotide sequence comparison of a wild type yellow-green (YG) click beetle luciferase nucleic acid sequence (LUCPLYG, SEQ ID NO:1) and the synthetic green click beetle luciferase nucleic acid sequences (GRver5.1, SEQ ID NO:9) and the synthetic red click beetle luciferase nucleic acid sequences (RD156-1H9, SEQ ID NO:18). The nucleotides enclosed in boxes are nucleotides that differ from the nucleotide present at the homologous



position in SEQ ID NO:1. Both synthetic sequences have a codon composition that differs from LUCPPLYG at more than 25% of the codons and have at least 3-fold fewer transcription regulatory sequences relative to a random selection of codons at the codons which differ.

5           Figure 12. An amino acid sequence comparison of a wild type YG click beetle luciferase amino acid sequence (LUCPPLYG, SEQ ID NO:23) and the synthetic GR click beetle luciferase amino acid sequences (GRver5.1, SEQ ID NO:31) and the red (RD) click beetle luciferase amino acid sequences (RD156-1H9, SEQ ID NO:223). All amino acid sequences are inferred from the  
10           corresponding nucleotide sequence. The amino acids enclosed in boxes are amino acids that differ from the amino acid present at the homologous position in SEQ ID NO:23.

          Figure 13. pRL vector series. All of the vectors contain the *Renilla* wild type or synthetic gene as further described herein. Figure 13A illustrates the  
15           *Renilla* luciferase gene in the pGL3 vectors (Promega Corp.) Figure 13B illustrates the *Renilla* luciferase co-reporter vector series. pRL-TK has the herpes simplex virus (HSV) tk promoter; pRL-SV40 has the SV40 virus early enhancer/promoter; pRL-CMV has the cytomegalovirus (CMV) enhancer and immediate early promoter; pRL-null has MCS (multiple cloning sites) but no  
20           promoter or enhancer; pRL-TK(Int<sup>-</sup>) has HSV/tk promoter without an intron that is present in the other plasmids; pR-GL3B has the pGL-3 Basic backbone (Promega Corp.); pR-GL3 TK has the pGL3-Basic backbone with an HSV tk promoter.

          Figure 14. Half-life of synthetic (Rluc-final) and native *Renilla*  
25           luciferases in CHO cells.

          Figures 15A-B. *In vitro* transcription/translation of *Renilla* luciferase nucleic acid sequences. A) t = 0-60 minutes; B) linear range.

          Figures 15C-D. *In vitro* translation of native and synthetic (Rluc-final) *Renilla* luciferase RNAs in a rabbit reticulocyte lysate. RNA was quantitated  
30           and the same amount was employed as in the translation reaction shown in Figures 15A-B. C) t = 0-60 minutes; D) linear range.

Figures 15E-F. Translation of native and synthetic (Rluc-final) *Renilla* RNAs in a wheat germ extract. E) t = 0-60 minutes; F) linear range.

Figure 16. High expression from a synthetic *Renilla* nucleic acid sequence reduces the risk of promoter interference in a co-transfection assay.

5 CHO cells were co-transfected with a constant amount (50 ng) of firefly luciferase expression vector (pGL3 control vector, with SV40 promoter and enhancer; Luc+) and a pRL vector having a native (0 ng, 50 ng, 100 ng, 500 ng, 1 µg or 2 µg) or synthetic (0 ng, 5 ng, 10 ng, 50 ng, 100 ng or 200 ng) *Renilla* luciferase gene.

10 Figures 17A-B. Illustrates the reactions catalyzed by firefly and click beetle (17A), and *Renilla* (17B) luciferases.

Figure 18. Nucleotide and inferred amino acid sequence of click beetle luciferases in pGL3 vectors (GRver5.1 in pGL3, SEQ ID NO:297 encoding SEQ ID NO:298; RDver5.1 in pGL3, SEQ ID NO:299 encoding SEQ ID NO:300; and  
15 RD156-1H9 in pGL3, SEQ ID NO:301 encoding SEQ ID NO:302). To clone GRver5.1, RDver5.1, and RD156-1H9 nucleic acid sequences into pGL3 vectors, an oligonucleotide having an *Nco* I site at the initiation codon was employed, which resulted in an amino acid substitution at position 2 to valine.

## 20 Detailed Description of the Invention

### Definitions

The term "gene" as used herein, refers to a DNA sequence that comprises coding sequences necessary for the production of a polypeptide or protein precursor. The polypeptide can be encoded by a full length coding sequence or  
25 by any portion of the coding sequence, as long as the desired protein activity is retained.

A "nucleic acid", as used herein, is a covalently linked sequence of nucleotides in which the 3' position of the pentose of one nucleotide is joined by a phosphodiester group to the 5' position of the pentose of the next, and in which  
30 the nucleotide residues (bases) are linked in specific sequence, i.e., a linear order of nucleotides. A "polynucleotide", as used herein, is a nucleic acid containing a sequence that is greater than about 100 nucleotides in length. An

"oligonucleotide", as used herein, is a short polynucleotide or a portion of a polynucleotide. An oligonucleotide typically contains a sequence of about two to about one hundred bases. The word "oligo" is sometimes used in place of the word "oligonucleotide".

5           Nucleic acid molecules are said to have a "5'-terminus" (5' end) and a "3'-terminus" (3' end) because nucleic acid phosphodiester linkages occur to the 5' carbon and 3' carbon of the pentose ring of the substituent mononucleotides. The end of a polynucleotide at which a new linkage would be to a 5' carbon is its 5' terminal nucleotide. The end of a polynucleotide at which a new linkage  
10       would be to a 3' carbon is its 3' terminal nucleotide. A terminal nucleotide, as used herein, is the nucleotide at the end position of the 3'- or 5'-terminus.

          DNA molecules are said to have "5' ends" and "3' ends" because mononucleotides are reacted to make oligonucleotides in a manner such that the 5' phosphate of one mononucleotide pentose ring is attached to the 3' oxygen of  
15       its neighbor in one direction via a phosphodiester linkage. Therefore, an end of an oligonucleotides referred to as the "5' end" if its 5' phosphate is not linked to the 3' oxygen of a mononucleotide pentose ring and as the "3' end" if its 3' oxygen is not linked to a 5' phosphate of a subsequent mononucleotide pentose ring.

20           As used herein, a nucleic acid sequence, even if internal to a larger oligonucleotide or polynucleotide, also may be said to have 5' and 3' ends. In either a linear or circular DNA molecule, discrete elements are referred to as being "upstream" or 5' of the "downstream" or 3' elements. This terminology reflects the fact that transcription proceeds in a 5' to 3' fashion along the DNA  
25       strand. Typically, promoter and enhancer elements that direct transcription of a linked gene are generally located 5' or upstream of the coding region. However, enhancer elements can exert their effect even when located 3' of the promoter element and the coding region. Transcription termination and polyadenylation signals are located 3' or downstream of the coding region.

30           The term "codon" as used herein, is a basic genetic coding unit, consisting of a sequence of three nucleotides that specify a particular amino acid to be incorporation into a polypeptide chain, or a start or stop signal. Figure 1

contains a codon table. The term "coding region" when used in reference to structural gene refers to the nucleotide sequences that encode the amino acids found in the nascent polypeptide as a result of translation of a mRNA molecule. Typically, the coding region is bounded on the 5' side by the nucleotide triplet "ATG" which encodes the initiator methionine and on the 3' side by a stop codon (e.g., TAA, TAG, TGA). In some cases the coding region is also known to initiate by a nucleotide triplet "TTG".

By "protein" and "polypeptide" is meant any chain of amino acids, regardless of length or post-translational modification (e.g., glycosylation or phosphorylation). The synthetic genes of the invention may also encode a variant of a naturally-occurring protein or polypeptide fragment thereof. Preferably, such a protein polypeptide has an amino acid sequence that is at least 85%, preferably 90%, and most preferably 95% or 99% identical to the amino acid sequence of the naturally-occurring (native) protein from which it is derived.

Polypeptide molecules are said to have an "amino terminus" (N-terminus) and a "carboxy terminus" (C-terminus) because peptide linkages occur between the backbone amino group of a first amino acid residue and the backbone carboxyl group of a second amino acid residue. The terms "N-terminal" and "C-terminal" in reference to polypeptide sequences refer to regions of polypeptides including portions of the N-terminal and C-terminal regions of the polypeptide, respectively. A sequence that includes a portion of the N-terminal region of polypeptide includes amino acids predominantly from the N-terminal half of the polypeptide chain, but is not limited to such sequences. For example, an N-terminal sequence may include an interior portion of the polypeptide sequence including bases from both the N-terminal and C-terminal halves of the polypeptide. The same applies to C-terminal regions. N-terminal and C-terminal regions may, but need not, include the amino acid defining the ultimate N-terminus and C-terminus of the polypeptide, respectively.

The term "wild type" as used herein, refers to a gene or gene product that has the characteristics of that gene or gene product isolated from a naturally

occurring source. A wild type gene is that which is most frequently observed in a population and is thus arbitrarily designated the "wild type" form of the gene.

In contrast, the term "mutant" refers to a gene or gene product that displays modifications in sequence and/or functional properties (i.e., altered

5 characteristics) when compared to the wild type gene or gene product. It is noted that naturally-occurring mutants can be isolated; these are identified by the fact that they have altered characteristics when compared to the wild type gene or gene product.

The terms "complementary" or "complementarity" are used in reference  
10 to a sequence of nucleotides related by the base-pairing rules. For example, for the sequence 5' "A-G-T" 3', is complementary to the sequence 3' "T-C-A" 5'. Complementarity may be "partial," in which only some of the nucleic acids' bases are matched according to the base pairing rules. Or, there may be "complete" or "total" complementarity between the nucleic acids. The degree of  
15 complementarity between nucleic acid strands has significant effects on the efficiency and strength of hybridization between nucleic acid strands. This is of particular importance in amplification reactions, as well as detection methods which depend upon hybridization of nucleic acids.

The term "recombinant protein" or "recombinant polypeptide" as used  
20 herein refers to a protein molecule expressed from a recombinant DNA molecule. In contrast, the term "native protein" is used herein to indicate a protein isolated from a naturally occurring (i.e., a nonrecombinant) source. Molecular biological techniques may be used to produce a recombinant form of a protein with identical properties as compared to the native form of the protein.

25 The terms "fusion protein" and "fusion partner" refer to a chimeric protein containing the protein of interest (e.g., luciferase) joined to an exogenous protein fragment (e.g., a fusion partner which consists of a non-luciferase protein). The fusion partner may enhance the solubility of protein as expressed in a host cell, may, for example, provide an affinity tag to allow purification of  
30 the recombinant fusion protein from the host cell or culture supernatant, or both. If desired, the fusion partner may be removed from the protein of interest by a variety of enzymatic or chemical means known to the art.

The terms "cell," "cell line," "host cell," as used herein, are used interchangeably, and all such designations include progeny or potential progeny of these designations. By "transformed cell" is meant a cell into which (or into an ancestor of which) has been introduced a DNA molecule comprising a synthetic gene. Optionally, a synthetic gene of the invention may be introduced into a suitable cell line so as to create a stably-transfected cell line capable of producing the protein or polypeptide encoded by the synthetic gene. Vectors, cells, and methods for constructing such cell lines are well known in the art, e.g. in Ausubel, et al. (*infra*). The words "transformants" or "transformed cells" include the primary transformed cells derived from the originally transformed cell without regard to the number of transfers. All progeny may not be precisely identical in DNA content, due to deliberate or inadvertent mutations. Nonetheless, mutant progeny that have the same functionality as screened for in the originally transformed cell are included in the definition of transformants.

Nucleic acids are known to contain different types of mutations. A "point" mutation refers to an alteration in the sequence of a nucleotide at a single base position from the wild type sequence. Mutations may also refer to insertion or deletion of one or more bases, so that the nucleic acid sequence differs from the wild-type sequence.

The term "homology" refers to a degree of complementarity. There may be partial homology or complete homology (i.e., identity). Homology is often measured using sequence analysis software (e.g., Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, WI 53705). Such software matches similar sequences by assigning degrees of homology to various substitutions, deletions, insertions, and other modifications. Conservative substitutions typically include substitutions within the following groups: glycine; alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid, asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine.

A "partially complementary" sequence is one that at least partially inhibits a completely complementary sequence from hybridizing to a target

nucleic acid is referred to using the functional term "substantially homologous."

The inhibition of hybridization of the completely complementary sequence to the target sequence may be examined using a hybridization assay (Southern or

Northern blot, solution hybridization and the like) under conditions of low

5 stringency. A substantially homologous sequence or probe will compete for and inhibit the binding (i.e., the hybridization) of a completely homologous to a

target under conditions of low stringency. This is not to say that conditions of low stringency are such that non-specific binding is permitted; low stringency conditions require that the binding of two sequences to one another be a specific

10 (i.e., selective) interaction. The absence of non-specific binding may be tested by the use of a second target which lacks even a partial degree of complementarity (e.g., less than about 30% identity). In this case, in the absence of non-specific binding, the probe will not hybridize to the second non-complementary target.

15 When used in reference to a double-stranded nucleic acid sequence such as a cDNA or a genomic clone, the term "substantially homologous" refers to any probe which can hybridize to either or both strands of the double-stranded nucleic acid sequence under conditions of low stringency as described herein.

"Probe" refers to an oligonucleotide designed to be sufficiently  
20 complementary to a sequence in a denatured nucleic acid to be probed (in relation to its length) to be bound under selected stringency conditions.

"Hybridization" and "binding" in the context of probes and denature melted nucleic acid are used interchangeably. Probes which are hybridized or bound to denatured nucleic acid are base paired to complementary sequences in  
25 the polynucleotide. Whether or not a particular probe remains base paired with the polynucleotide depends on the degree of complementarity, the length of the probe, and the stringency of the binding conditions. The higher the stringency, the higher must be the degree of complementarity and/or the longer the probe.

The term "hybridization" is used in reference to the pairing of  
30 complementary nucleic acid strands. Hybridization and the strength of hybridization (i.e., the strength of the association between nucleic acid strands) is impacted by many factors well known in the art including the degree of

complementarity between the nucleic acids, stringency of the conditions involved affected by such conditions as the concentration of salts, the  $T_m$  (melting temperature) of the formed hybrid, the presence of other components (e.g., the presence or absence of polyethylene glycol), the molarity of the hybridizing strands and the G:C content of the nucleic acid strands.

The term "stringency" is used in reference to the conditions of temperature, ionic strength, and the presence of other compounds, under which nucleic acid hybridizations are conducted. With "high stringency" conditions, nucleic acid base pairing will occur only between nucleic acid fragments that have a high frequency of complementary base sequences. Thus, conditions of "medium" or "low" stringency are often required when it is desired that nucleic acids which are not completely complementary to one another be hybridized or annealed together. The art knows well that numerous equivalent conditions can be employed to comprise medium or low stringency conditions. The choice of hybridization conditions is generally evident to one skilled in the art and is usually guided by the purpose of the hybridization, the type of hybridization (DNA-DNA or DNA-RNA), and the level of desired relatedness between the sequences (e.g., Sambrook et al., 1989; Nucleic Acid Hybridization, A Practical Approach, IRL Press, Washington D.C., 1985, for a general discussion of the methods).

The stability of nucleic acid duplexes is known to decrease with an increased number of mismatched bases, and further to be decreased to a greater or lesser degree depending on the relative positions of mismatches in the hybrid duplexes. Thus, the stringency of hybridization can be used to maximize or minimize stability of such duplexes. Hybridization stringency can be altered by: adjusting the temperature of hybridization; adjusting the percentage of helix destabilizing agents, such as formamide, in the hybridization mix; and adjusting the temperature and/or salt concentration of the wash solutions. For filter hybridizations, the final stringency of hybridizations often is determined by the salt concentration and/or temperature used for the post-hybridization washes.

"High stringency conditions" when used in reference to nucleic acid hybridization comprise conditions equivalent to binding or hybridization at 42°C



in a solution consisting of 5X SSPE (43.8 g/l NaCl, 6.9 g/l NaH<sub>2</sub>PO<sub>4</sub> H<sub>2</sub>O and 1.85 g/l EDTA, pH adjusted to 7.4 with NaOH), 0.5% SDS, 5X Denhardt's reagent and 100 µg/ml denatured salmon sperm DNA followed by washing in a solution comprising 0.1X SSPE, 1.0% SDS at 42°C when a probe of about 500 nucleotides in length is employed.

"Medium stringency conditions" when used in reference to nucleic acid hybridization comprise conditions equivalent to binding or hybridization at 42°C in a solution consisting of 5X SSPE (43.8 g/l NaCl, 6.9 g/l NaH<sub>2</sub>PO<sub>4</sub> H<sub>2</sub>O and 1.85 g/l EDTA, pH adjusted to 7.4 with NaOH), 0.5% SDS, 5X Denhardt's reagent and 100 µg/ml denatured salmon sperm DNA followed by washing in a solution comprising 1.0X SSPE, 1.0% SDS at 42°C when a probe of about 500 nucleotides in length is employed.

"Low stringency conditions" comprise conditions equivalent to binding or hybridization at 42°C in a solution consisting of 5X SSPE (43.8 g/l NaCl, 6.9 g/l NaH<sub>2</sub>PO<sub>4</sub> H<sub>2</sub>O and 1.85 g/l EDTA, pH adjusted to 7.4 with NaOH), 0.1% SDS, 5X Denhardt's reagent [50X Denhardt's contains per 500 ml: 5 g Ficoll (Type 400, Pharmacia), 5 g BSA (Fraction V; Sigma)] and 100 g/ml denatured salmon sperm DNA followed by washing in a solution comprising 5X SSPE, 0.1% SDS at 42°C when a probe of about 500 nucleotides in length is employed.

The term "T<sub>m</sub>" is used in reference to the "melting temperature". The melting temperature is the temperature at which 50% of a population of double-stranded nucleic acid molecules becomes dissociated into single strands. The equation for calculating the T<sub>m</sub> of nucleic acids is well-known in the art. The T<sub>m</sub> of a hybrid nucleic acid is often estimated using a formula adopted from hybridization assays in 1 M salt, and commonly used for calculating T<sub>m</sub> for PCR primers: [(number of A + T) x 2°C + (number of G+C) x 4°C]. (C.R. Newton et al., PCR, 2nd Ed., Springer-Verlag (New York, 1997), p. 24). This formula was found to be inaccurate for primers longer than 20 nucleotides. (Id.) Another simple estimate of the T<sub>m</sub> value may be calculated by the equation: T<sub>m</sub> = 81.5 + 0.41(% G + C), when a nucleic acid is in aqueous solution at 1 M NaCl. (e.g., Anderson and Young, Quantitative Filter Hybridization, in Nucleic Acid Hybridization, 1985). Other more sophisticated computations exist in the art

which take structural as well as sequence characteristics into account for the calculation of  $T_m$ . A calculated  $T_m$  is merely an estimate; the optimum temperature is commonly determined empirically.

The term "isolated" when used in relation to a nucleic acid, as in "isolated oligonucleotide" or "isolated polynucleotide" refers to a nucleic acid sequence that is identified and separated from at least one contaminant with which it is ordinarily associated in its source. Thus, an isolated nucleic acid is present in a form or setting that is different from that in which it is found in nature. In contrast, non-isolated nucleic acids (e.g., DNA and RNA) are found in the state they exist in nature. For example, a given DNA sequence (e.g., a gene) is found on the host cell chromosome in proximity to neighboring genes; RNA sequences (e.g., a specific mRNA sequence encoding a specific protein), are found in the cell as a mixture with numerous other mRNAs that encode a multitude of proteins. However, isolated nucleic acid includes, by way of example, such nucleic acid in cells ordinarily expressing that nucleic acid where the nucleic acid is in a chromosomal location different from that of natural cells, or is otherwise flanked by a different nucleic acid sequence than that found in nature. The isolated nucleic acid or oligonucleotide may be present in single-stranded or double-stranded form. When an isolated nucleic acid or oligonucleotide is to be utilized to express a protein, the oligonucleotide contains at a minimum, the sense or coding strand (i.e., the oligonucleotide may single-stranded), but may contain both the sense and anti-sense strands (i.e., the oligonucleotide may be double-stranded).

The term "isolated" when used in relation to a polypeptide, as in "isolated protein" or "isolated polypeptide" refers to a polypeptide that is identified and separated from at least one contaminant with which it is ordinarily associated in its source. Thus, an isolated polypeptide is present in a form or setting that is different from that in which it is found in nature. In contrast, non-isolated polypeptides (e.g., proteins and enzymes) are found in the state they exist in nature.

The term "purified" or "to purify" means the result of any process that removes some of a contaminant from the component of interest, such as a protein

or nucleic acid. The percent of a purified component is thereby increased in the sample.

The term "operably linked" as used herein refer to the linkage of nucleic acid sequences in such a manner that a nucleic acid molecule capable of directing the transcription of a given gene and/or the synthesis of a desired protein molecule is produced. The term also refers to the linkage of sequences encoding amino acids in such a manner that a functional (e.g., enzymatically active, capable of binding to a binding partner, capable of inhibiting, etc.) protein or polypeptide is produced.

The term "recombinant DNA molecule" means a hybrid DNA sequence comprising at least two nucleotide sequences not normally found together in nature.

The term "vector" is used in reference to nucleic acid molecules into which fragments of DNA may be inserted or cloned and can be used to transfer DNA segment(s) into a cell and capable of replication in a cell. Vectors may be derived from plasmids, bacteriophages, viruses, cosmids, and the like.

The terms "recombinant vector" and "expression vector" as used herein refer to DNA or RNA sequences containing a desired coding sequence and appropriate DNA or RNA sequences necessary for the expression of the operably linked coding sequence in a particular host organism. Prokaryotic expression vectors include a promoter, a ribosome binding site, an origin of replication for autonomous replication in a host cell and possibly other sequences, e.g. an optional operator sequence, optional restriction enzyme sites. A promoter is defined as a DNA sequence that directs RNA polymerase to bind to DNA and to initiate RNA synthesis. Eukaryotic expression vectors include a promoter, optionally a polyadenylation signal and optionally an enhancer sequence.

The term "a polynucleotide having a nucleotide sequence encoding a gene," means a nucleic acid sequence comprising the coding region of a gene, or in other words the nucleic acid sequence which encodes a gene product. The coding region may be present in either a cDNA, genomic DNA or RNA form. When present in a DNA form, the oligonucleotide may be single-stranded (i.e., the sense strand) or double-stranded. Suitable control elements such as enhancers/promoters, splice junctions, polyadenylation signals, etc. may be

placed in close proximity to the coding region of the gene if needed to permit proper initiation of transcription and/or correct processing of the primary RNA transcript. Alternatively, the coding region utilized in the expression vectors of the present invention may contain endogenous enhancers/promoters, splice  
5 junctions, intervening sequences, polyadenylation signals, etc. In further embodiments, the coding region may contain a combination of both endogenous and exogenous control elements.

The term "transcription regulatory element" or "transcription regulatory sequence" refers to a genetic element or sequence that controls some aspect of  
10 the expression of nucleic acid sequence(s). For example, a promoter is a regulatory element that facilitates the initiation of transcription of an operably linked coding region. Other regulatory elements include, but are not limited to, transcription factor binding sites, splicing signals, polyadenylation signals, termination signals and enhancer elements.

15 Transcriptional control signals in eukaryotes comprise "promoter" and "enhancer" elements. Promoters and enhancers consist of short arrays of DNA sequences that interact specifically with cellular proteins involved in transcription (Maniatis et al., 1987). Promoter and enhancer elements have been isolated from a variety of eukaryotic sources including genes in yeast, insect and  
20 mammalian cells. Promoter and enhancer elements have also been isolated from viruses and analogous control elements, such as promoters, are also found in prokaryotes. The selection of a particular promoter and enhancer depends on the cell type used to express the protein of interest. Some eukaryotic promoters and enhancers have a broad host range while others are functional in a limited subset  
25 of cell types (for review, see Voss et al., 1986; and Maniatis et al., 1987. For example, the SV40 early gene enhancer is very active in a wide variety of cell types from many mammalian species and has been widely used for the expression of proteins in mammalian cells (Dijkema et al., 1985). Two other examples of promoter/enhancer elements active in a broad range of mammalian  
30 cell types are those from the human elongation factor 1 gene (Uetsuki et al., 1989; Kim, et al., 1990; and Mizushima and Nagata, 1990) and the long terminal

repeats of the Rous sarcoma virus (Gorman et al., 1982); and the human cytomegalovirus (Boshart et al., 1985).

The term "promoter/enhancer" denotes a segment of DNA containing sequences capable of providing both promoter and enhancer functions (i.e., the functions provided by a promoter element and an enhancer element as described above). For example, the long terminal repeats of retroviruses contain both promoter and enhancer functions. The enhancer/promoter may be "endogenous" or "exogenous" or "heterologous." An "endogenous" enhancer/promoter is one that is naturally linked with a given gene in the genome. An "exogenous" or "heterologous" enhancer/promoter is one that is placed in juxtaposition to a gene by means of genetic manipulation (i.e., molecular biological techniques) such that transcription of the gene is directed by the linked enhancer/promoter.

The presence of "splicing signals" on an expression vector often results in higher levels of expression of the recombinant transcript in eukaryotic host cells.

Splicing signals mediate the removal of introns from the primary RNA transcript and consist of a splice donor and acceptor site (Sambrook, et al., Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, New York, 1989, pp. 16.7-16.8). A commonly used splice donor and acceptor site is the splice junction from the 16S RNA of SV40.

Efficient expression of recombinant DNA sequences in eukaryotic cells requires expression of signals directing the efficient termination and polyadenylation of the resulting transcript. Transcription termination signals are generally found downstream of the polyadenylation signal and are a few hundred nucleotides in length. The term "poly(A) site" or "poly(A) sequence" as used herein denotes a DNA sequence which directs both the termination and polyadenylation of the nascent RNA transcript. Efficient polyadenylation of the recombinant transcript is desirable, as transcripts lacking a poly(A) tail are unstable and are rapidly degraded. The poly(A) signal utilized in an expression vector may be "heterologous" or "endogenous." An endogenous poly(A) signal is one that is found naturally at the 3' end of the coding region of a given gene in the genome. A heterologous poly(A) signal is one which has been isolated from one gene and positioned 3' to another gene. A commonly used heterologous

poly(A) signal is the SV40 poly(A) signal. The SV40 poly(A) signal is contained on a 237 bp *Bam*H I/*Bcl* I restriction fragment and directs both termination and polyadenylation (Sambrook, supra, at 16.6-16.7).

Eukaryotic expression vectors may also contain "viral replicons" or "viral origins of replication." Viral replicons are viral DNA sequences which allow for the extrachromosomal replication of a vector in a host cell expressing the appropriate replication factors. Vectors containing either the SV40 or polyoma virus origin of replication replicate to high copy number (up to  $10^4$  copies/cell) in cells that express the appropriate viral T antigen. In contrast, vectors containing the replicons from bovine papillomavirus or Epstein-Barr virus replicate extrachromosomally at low copy number (about 100 copies/cell).

The term "*in vitro*" refers to an artificial environment and to processes or reactions that occur within an artificial environment. *In vitro* environments include, but are not limited to, test tubes and cell lysates. The term "*in situ*" refers to cell culture. The term "*in vivo*" refers to the natural environment (e.g., an animal or a cell) and to processes or reaction that occur within a natural environment.

The term "expression system" refers to any assay or system for determining (e.g., detecting) the expression of a gene of interest. Those skilled in the field of molecular biology will understand that any of a wide variety of expression systems may be used. A wide range of suitable mammalian cells are available from a wide range of source (e.g., the American Type Culture Collection, Rockland, MD). The method of transformation or transfection and the choice of expression vehicle will depend on the host system selected. Transformation and transfection methods are described, e.g., in Ausubel, et al., Current Protocols in Molecular Biology. John Wiley & Sons, New York. 1992. Expression systems include *in vitro* gene expression assays where a gene of interest (e.g., a reporter gene) is linked to a regulatory sequence and the expression of the gene is monitored following treatment with an agent that inhibits or induces expression of the gene. Detection of gene expression can be through any suitable means including, but not limited to, detection of expressed mRNA or protein (e.g., a detectable product of a reporter gene) or through a

detectable change in the phenotype of a cell expressing the gene of interest. Expression systems may also comprise assays where a cleavage event or other nucleic acid or cellular change is detected.

5 The term "enzyme" refers to molecules or molecule aggregates that are responsible for catalyzing chemical and biological reactions. Such molecules are typically proteins, but can also comprise short peptides, RNAs, ribozymes, antibodies, and other molecules. A molecule that catalyzes chemical and biological reactions is referred to as "having enzyme activity" or "having catalytic activity."

10 All amino acid residues identified herein are in the natural L-configuration. In keeping with standard polypeptide nomenclature (see J. Biol. Chem., 243, 3557 (1969)), abbreviations for amino acid residues are as shown in the following Table of Correspondence.

15 TABLE OF CORRESPONDENCE

|    | 1-Letter | 3-Letter | AMINO ACID      |
|----|----------|----------|-----------------|
|    | Y        | Tyr      | L-tyrosine      |
|    | G        | Gly      | glycine         |
|    | F        | Phe      | L-phenylalanine |
| 20 | M        | Met      | L-methionine    |
|    | A        | Ala      | L-alanine       |
|    | S        | Ser      | L-serine        |
|    | I        | Ile      | L-isoleucine    |
|    | L        | Leu      | L-leucine       |
| 25 | T        | Thr      | L-threonine     |
|    | V        | Val      | L-valine        |
|    | P        | Pro      | L-proline       |
|    | K        | Lys      | L-lysine        |
|    | H        | His      | L-histidine     |
| 30 | Q        | Gln      | L-glutamine     |
|    | E        | Glu      | L-glutamic acid |
|    | W        | Trp      | L-tryptophan    |

|   |     |                 |
|---|-----|-----------------|
| R | Arg | L-arginine      |
| D | Asp | L-aspartic acid |
| N | Asn | L-asparagine    |
| C | Cys | L-cysteine      |

5

The term "sequence homology" means the proportion of base matches between two nucleic acid sequences or the proportion of amino acid matches between two amino acid sequences. When sequence homology is expressed as a percentage, e.g., 50%, the percentage denotes the proportion of matches over the length of sequence from one sequence that is compared to some other sequence. Gaps (in either of the two sequences) are permitted to maximize matching; gap lengths of 15 bases or less are usually used, 6 bases or less are preferred with 2 bases or less more preferred. When using oligonucleotides as probes or treatments, the sequence homology between the target nucleic acid and the oligonucleotide sequence is generally not less than 17 target base matches out of 20 possible oligonucleotide base pair matches (85%); preferably not less than 9 matches out of 10 possible base pair matches (90%), and more preferably not less than 19 matches out of 20 possible base pair matches (95%).

Two amino acid sequences are homologous if there is a partial or complete identity between their sequences. For example, 85% homology means that 85% of the amino acids are identical when the two sequences are aligned for maximum matching. Gaps (in either of the two sequences being matched) are allowed in maximizing matching; gap lengths of 5 or less are preferred with 2 or less being more preferred. Alternatively and preferably, two protein sequences (or polypeptide sequences derived from them of at least 100 amino acids in length) are homologous, as this term is used herein, if they have an alignment score of at more than 5 (in standard deviation units) using the program ALIGN with the mutation data matrix and a gap penalty of 6 or greater. See Dayhoff, M. O., in Atlas of Protein Sequence and Structure, 1972, volume 5, National Biomedical Research Foundation, pp. 101-110, and Supplement 2 to this volume, pp. 1-10. The two sequences or parts thereof are more preferably



homologous if their amino acids are greater than or equal to 85% identical when optimally aligned using the ALIGN program.

The following terms are used to describe the sequence relationships between two or more polynucleotides: "reference sequence", "comparison window", "sequence identity", "percentage of sequence identity", and "substantial identity". A "reference sequence" is a defined sequence used as a basis for a sequence comparison; a reference sequence may be a subset of a larger sequence, for example, as a segment of a full-length cDNA or gene sequence given in a sequence listing, or may comprise a complete cDNA or gene sequence. Generally, a reference sequence is at least 20 nucleotides in length, frequently at least 25 nucleotides in length, and often at least 50 nucleotides in length. Since two polynucleotides may each (1) comprise a sequence (i.e., a portion of the complete polynucleotide sequence) that is similar between the two polynucleotides, and (2) may further comprise a sequence that is divergent between the two polynucleotides, sequence comparisons between two (or more) polynucleotides are typically performed by comparing sequences of the two polynucleotides over a "comparison window" to identify and compare local regions of sequence similarity.

A "comparison window", as used herein, refers to a conceptual segment of at least 20 contiguous nucleotides and wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (i.e., gaps) of 20 percent or less as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences.

Methods of alignment of sequences for comparison are well known in the art. Thus, the determination of percent identity between any two sequences can be accomplished using a mathematical algorithm. Preferred, non-limiting examples of such mathematical algorithms are the algorithm of Myers and Miller (1988); the local homology algorithm of Smith and Waterman (1981); the homology alignment algorithm of Needleman and Wunsch (1970); the search-for-similarity-method of Pearson and Lipman (1988); the algorithm of Karlin and Altschul (1990), modified as in Karlin and Altschul (1993).

Computer implementations of these mathematical algorithms can be utilized for comparison of sequences to determine sequence identity. Such implementations include, but are not limited to: CLUSTAL in the PC/Gene program (available from Intelligenetics, Mountain View, California); the ALIGN  
5 program (Version 2.0) and GAP, BESTFIT, BLAST, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Version 8 (available from Genetics Computer Group (GCG), 575 Science Drive, Madison, Wisconsin, USA).

Alignments using these programs can be performed using the default parameters.

The CLUSTAL program is well described by Higgins et al. (1988); Higgins et al. (1989); Corpet et al. (1988); Huang et al. (1992); and Pearson et al. (1994).  
10 The ALIGN program is based on the algorithm of Myers and Miller, *supra*. The BLAST programs of Altschul et al. (1990), are based on the algorithm of Karlin and Altschul *supra*. To obtain gapped alignments for comparison purposes, Gapped BLAST (in BLAST 2.0) can be utilized as described in Altschul et al.  
15 (1997). Alternatively, PSI-BLAST (in BLAST 2.0) can be used to perform an iterated search that detects distant relationships between molecules. See Altschul et al., *supra*. When utilizing BLAST, Gapped BLAST, PSI-BLAST, the default parameters of the respective programs (e.g. BLASTN for nucleotide sequences, BLASTX for proteins) can be used. See  
20 <http://www.ncbi.nlm.nih.gov>. Alignment may also be performed manually by inspection.

The term "sequence identity" means that two polynucleotide sequences are identical (i.e., on a nucleotide-by-nucleotide basis) over the window of comparison. The term "percentage of sequence identity" means that two  
25 polynucleotide sequences are identical (i.e., on a nucleotide-by-nucleotide basis) for the stated proportion of nucleotides over the window of comparison. The term "percentage of sequence identity" is calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical nucleic acid base (e.g., A, T, C, G, U, or I)  
30 occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison (i.e., the window size), and multiplying the result by 100 to yield the

percentage of sequence identity. The terms "substantial identity" as used herein denote a characteristic of a polynucleotide sequence, wherein the polynucleotide comprises a sequence that has at least 60%, preferably at least 65%, more preferably at least 70%, up to about 85%, and even more preferably at least 90 to 5 95%, more usually at least 99%, sequence identity as compared to a reference sequence over a comparison window of at least 20 nucleotide positions, frequently over a window of at least 20-50 nucleotides, and preferably at least 300 nucleotides, wherein the percentage of sequence identity is calculated by comparing the reference sequence to the polynucleotide sequence which may 10 include deletions or additions which total 20 percent or less of the reference sequence over the window of comparison. The reference sequence may be a subset of a larger sequence.

As applied to polypeptides, the term "substantial identity" means that two peptide sequences, when optimally aligned, such as by the programs GAP or 15 BESTFIT using default gap weights, share at least about 85% sequence identity, preferably at least about 90% sequence identity, more preferably at least about 95 % sequence identity, and most preferably at least about 99 % sequence identity.

## 20 The Synthetic Nucleic Acid Molecules and Methods of the Invention

The invention provides compositions comprising synthetic nucleic acid molecules, as well as methods for preparing those molecules which yield synthetic nucleic acid molecules that are efficiently expressed as a polypeptide or protein with desirable characteristics including reduced inappropriate or 25 unintended transcription characteristics when expressed in a particular cell type.

Natural selection is the hypothesis that genotype-environment interactions occurring at the phenotypic level lead to differential reproductive success of individuals and hence to modification of the gene pool of a population. It is generally accepted that the amino acid sequence of a protein 30 found in nature has undergone optimization by natural selection. However, amino acids exist within the sequence of a protein that do not contribute significantly to the activity of the protein and these amino acids can be changed

to other amino acids with little or no consequence. Furthermore, a protein may be useful outside its natural environment or for purposes that differ from the conditions of its natural selection. In these circumstances, the amino acid sequence can be synthetically altered to better adapt the protein for its utility in various applications.

Likewise, the nucleic acid sequence that encodes a protein is also optimized by natural selection. The relationship between coding DNA and its transcribed RNA is such that any change to the DNA affects the resulting RNA. Thus, natural selection works on both molecules simultaneously. However, this relationship does not exist between nucleic acids and proteins. Because multiple codons encode the same amino acid, many different nucleotide sequences can encode an identical protein. A specific protein composed of 500 amino acids can theoretically be encoded by more than  $10^{150}$  different nucleic acid sequences.

Natural selection acts on nucleic acids to achieve proper encoding of the corresponding protein. Presumably, other properties of nucleic acid molecules are also acted upon by natural selection. These properties include codon usage frequency, RNA secondary structure, the efficiency of intron splicing, and interactions with transcription factors or other nucleic acid binding proteins. These other properties may alter the efficiency of protein translation and the resulting phenotype. Because of the redundant nature of the genetic code, these other attributes can be optimized by natural selection without altering the corresponding amino acid sequence.

Under some conditions, it is useful to synthetically alter the natural nucleotide sequence encoding a protein to better adapt the protein for alternative applications. A common example is to alter the codon usage frequency of a gene when it is expressed in a foreign host. Although redundancy in the genetic code allows amino acids to be encoded by multiple codons, different organisms favor some codons over others. The codon usage frequencies tend to differ most for organisms with widely separated evolutionary histories. It has been found that when transferring genes between evolutionarily distant organisms, the efficiency of protein translation can be substantially increased by adjusting the codon usage frequency (see U.S. Patent Nos. 5,096,825, 5,670,356 and 5,874,304).

Because of the need for evolutionary distance, the codon usage of reporter genes often does not correspond to the optimal codon usage of the experimental cells. Examples include  $\beta$ -galactosidase ( $\beta$ -gal) and chloramphenicol acetyltransferase (*cat*) reporter genes that are derived from *E. coli* and are commonly used in mammalian cells; the  $\beta$ -glucuronidase (*gus*) reporter gene that is derived from *E. coli* and commonly used in plant cells; the firefly luciferase (*luc*) reporter gene that is derived from an insect and commonly used in plant and mammalian cells; and the *Renilla* luciferase, and green fluorescent protein (*gfp*) reporter genes which are derived from coelenterates and are commonly used in plant and mammalian cells. To achieve sensitive quantitation of reporter gene expression, the activity of the gene product must not be endogenous to the experimental host cells. Thus, reporter genes are usually selected from organisms having unique and distinctive phenotypes. Consequently, these organisms often have widely separated evolutionary histories from the experimental host cells.

Previously, to create genes having a more optimal codon usage frequency but still encoding the same gene product, a synthetic nucleic acid sequence was made by replacing existing codons with codons that were generally more favorable to the experimental host cell (see U.S. Patent Nos. 5,096,825, 5,670,356 and 5,874,304.) The result was a net improvement in codon usage frequency of the synthetic gene. However, the optimization of other attributes was not considered and so these synthetic genes likely did not reflect genes optimized by natural selection.

In particular, improvements in codon usage frequency are intended only for optimization of a RNA sequence based on its role in translation into a protein. Thus, previously described methods did not address how the sequence of a synthetic gene affects the role of DNA in transcription into RNA. Most notably, consideration had not been given as to how transcription factors may interact with the synthetic DNA and consequently modulate or otherwise influence gene transcription. For genes found in nature, the DNA would be optimally transcribed by the native host cell and would yield an RNA that encodes a properly folded gene product. In contrast, synthetic genes have

previously not been optimized for transcriptional characteristics. Rather, this property has been ignored or left to chance.

This concern is important for all genes, but particularly important for reporter genes, which are most commonly used to quantitate transcriptional behavior in the experimental host cells. Hundreds of transcription factors have been identified in different cell types under different physiological conditions, and likely more exist but have not yet been identified. All of these transcription factors can influence the transcription of an introduced gene. A useful synthetic reporter gene of the invention has a minimal risk of influencing or perturbing intrinsic transcriptional characteristics of the host cell because the structure of that gene has been altered. A particularly useful synthetic reporter gene will have desirable characteristics under a new set and/or a wide variety of experimental conditions. To best achieve these characteristics, the structure of the synthetic gene should have minimal potential for interacting with transcription factors within a broad range of host cells and physiological conditions. Minimizing potential interactions between a reporter gene and a host cell's endogenous transcription factors increases the value of a reporter gene by reducing the risk of inappropriate transcriptional characteristics of the gene within a particular experiment, increasing applicability of the gene in various environments, and increasing the acceptance of the resulting experimental data.

In contrast, a reporter gene comprising a native nucleotide sequence, based on a genomic or cDNA clone from the original host organism, may interact with transcription factors when expressed in an exogenous host. This risk stems from two circumstances. First, the native nucleotide sequence contains sequences that were optimized through natural selection to influence gene transcription within the native host organism. However, these sequences might also influence transcription when the gene is expressed in exogenous hosts, i.e., out of context, thus interfering with its performance as a reporter gene. Second, the nucleotide sequence may inadvertently interact with transcription factors that were not present in the native host organism, and thus did not participate in its natural selection. The probability of such inadvertent

interactions increases with greater evolutionary separation between the experimental cells and the native organism of the reporter gene.

These potential interactions with transcription factors would likely be disrupted when using a synthetic reporter gene having alterations in codon usage frequency. However, a synthetic reporter gene sequence, designed by choosing codons based only on codon usage frequency, is likely to contain other unintended transcription factor binding sites since the synthetic gene has not been subjected to the benefit of natural selection to correct inappropriate transcriptional activities. Inadvertent interactions with transcription factors could also occur whenever the encoded amino acid sequence is artificially altered, e.g., to introduce amino acid substitutions. Similarly, these changes have not been subjected to natural selection, and thus may exhibit undesired characteristics.

Thus, the invention provides a method for preparing synthetic nucleic acid sequences that reduce the risk of undesirable interactions of the nucleic acid with transcription factors when expressed in a particular host cell, thereby reducing inappropriate or unintended transcriptional characteristics. Preferably, the method yields synthetic genes containing improved codon usage frequencies for a particular host cell and with a reduced occurrence of transcription factor binding sites. The invention also provides a method of preparing synthetic genes containing improved codon usage frequencies with a reduced occurrence of transcription factor binding sites and additional beneficial structural attributes.

Such additional attributes include the absence of inappropriate RNA splicing junctions, poly(A) addition signals, undesirable restriction sites, ribosomal binding sites, and secondary structural motifs such as hairpin loops.

Also provided is a method for preparing two synthetic genes encoding the same or highly similar proteins ("codon distinct" versions). Preferably, the two synthetic genes have a reduced ability to hybridize to a common polynucleotide probe sequence, or have a reduced risk of recombining when present together in living cells. To detect recombination, PCR amplification of the reporter sequences using primers complementary to flanking sequences and sequencing of the amplified sequences may be employed.

To select codons for the synthetic nucleic acid molecules of the invention, preferred codons have a relatively high codon usage frequency in a selected host cell, and their introduction results in the introduction of relatively few transcription factor binding sites, relatively few other undesirable structural attributes, and optionally a characteristic that distinguishes the synthetic gene from another gene encoding a highly similar protein. Thus, the synthetic nucleic acid product obtained by the method of the invention is a synthetic gene with improved level of expression due to improved codon usage frequency, a reduced risk of inappropriate transcriptional behavior due to a reduced number of undesirable transcription regulatory sequences, and optionally any additional characteristic due to other criteria that may be employed to select the synthetic sequence.

The invention may be employed with any nucleic acid sequence, e.g., a native sequence such as a cDNA or one which has been manipulated *in vitro*, e.g., to introduce specific alterations such as the introduction or removal of a restriction enzyme recognition site, the alteration of a codon to encode a different amino acid or to encode a fusion protein, or to alter GC or AT content (% of composition) of nucleic acid molecules. Moreover, the method of the invention is useful with any gene, but particularly useful for reporter genes as well as other genes associated with the expression of reporter genes, such as selectable markers. Preferred genes include, but are not limited to, those encoding lactamase ( $\beta$ -gal), neomycin resistance (Neo), CAT, GUS, galactopyranoside, GFP, xylosidase, thymidine kinase, arabinosidase and the like. As used herein, a "marker gene" or "reporter gene" is a gene that imparts a distinct phenotype to cells expressing the gene and thus permits cells having the gene to be distinguished from cells that do not have the gene. Such genes may encode either a selectable or screenable marker, depending on whether the marker confers a trait which one can 'select' for by chemical means, i.e., through the use of a selective agent (e.g., a herbicide, antibiotic, or the like), or whether it is simply a "reporter" trait that one can identify through observation or testing, i.e., by 'screening'. Elements of the present disclosure are exemplified in detail through the use of particular marker genes. Of course, many examples of



suitable marker genes or reporter genes are known to the art and can be employed in the practice of the invention. Therefore, it will be understood that the following discussion is exemplary rather than exhaustive. In light of the techniques disclosed herein and the general recombinant techniques which are known in the art, the present invention renders possible the alteration of any gene.

Exemplary marker genes include, but are not limited to, a *neo* gene, a  $\beta$ -gal gene, a *gus* gene, a *cat* gene, a *gpt* gene, a *hyg* gene, a *hisD* gene, a *ble* gene, a *mprr* gene, a *bar* gene, a nitrilase gene, a mutant acetolactate synthase gene (ALS) or acetoacid synthase gene (AAS), a methotrexate-resistant *dhfr* gene, a dalapon dehalogenase gene, a mutated anthranilate synthase gene that confers resistance to 5-methyl tryptophan (WO 97/26366), an R-locus gene, a  $\beta$ -lactamase gene, a *xylE* gene, an  $\alpha$ -amylase gene, a tyrosinase gene, a luciferase (*luc*) gene, (e.g., a *Renilla reniformis* luciferase gene, a firefly luciferase gene, or a click beetle luciferase (*Pyrophorus plagiophthalmus*) gene), an aequorin gene, or a green fluorescent protein gene. Included within the terms selectable or screenable marker genes are also genes which encode a "secretable marker" whose secretion can be detected as a means of identifying or selecting for transformed cells. Examples include markers which encode a secretable antigen that can be identified by antibody interaction, or even secretable enzymes which can be detected by their catalytic activity. Secretable proteins fall into a number of classes, including small, diffusible proteins detectable, e.g., by ELISA, and proteins that are inserted or trapped in the cell membrane.

The method of the invention can be performed by, although it is not limited to, a recursive process. The process includes assigning preferred codons to each amino acid in a target molecule, e.g., a native nucleotide sequence, based on codon usage in a particular species, identifying potential transcription regulatory sequences such as transcription factor binding sites in the nucleic acid sequence having preferred codons, e.g., using a database of such binding sites, optionally identifying other undesirable sequences, and substituting an alternative codon (i.e., encoding the same amino acid) at positions where undesirable transcription factor binding sites or other sequences occur. For

codon distinct versions, alternative preferred codons are substituted in each version. If necessary, the identification and elimination of potential transcription factor or other undesirable sequences can be repeated until a nucleotide sequence is achieved containing a maximum number of preferred codons and a minimum  
5 number of undesired sequences including transcription regulatory sequences or other undesirable sequences. Also, optionally, desired sequences, e.g., restriction enzyme recognition sites, can be introduced. After a synthetic nucleic acid molecule is designed and constructed, its properties relative to the parent nucleic acid sequence can be determined by methods well known to the art. For  
10 example, the expression of the synthetic and target nucleic acid molecules in a series of vectors in a particular cell can be compared.

Thus, generally, the method of the invention comprises identifying a target nucleic acid sequence, such as a vector backbone, a reporter gene or a selectable marker gene, and a host cell of interest, for example, a plant (dicot or  
15 monocot), fungus, yeast or mammalian cell. Preferred host cells are mammalian host cells such as CHO, COS, 293, HeLa, CV-1 and NIH3T3 cells. Based on preferred codon usage in the host cell(s) and, optionally, low codon usage in the host cell(s), e.g., high usage mammalian codons and low usage *E. coli* and mammalian codons, codons to be replaced are determined. For codon distinct  
20 versions of two synthetic nucleic acid molecules, alternative preferred codons are introduced to each version. Thus, for amino acids having more than two codons, one preferred codon is introduced to one version and another preferred codon is introduced to the other version. For amino acids having six codons, the two codons with the largest number of mismatched bases are identified and one is  
25 introduced to one version and the other codon is introduced to the other version. Concurrent, subsequent or prior to selecting codons to be replaced, desired and undesired sequences, such as undesired transcriptional regulatory sequences, in the target sequence are identified. These sequences can be identified using databases and software such as EPD, NNPD, REBASE, TRANSFAC, TESS,  
30 GenePro, MAR ([www.ncgr.org/MAR-search](http://www.ncgr.org/MAR-search)) and BCM Gene Finder, further described herein. After the sequences are identified, the modification(s) are introduced. Once a desired synthetic nucleic acid sequence is obtained, it can be

prepared by methods well known to the art (such as PCR with overlapping primers), and its structural and functional properties compared to the target nucleic acid sequence, including, but not limited to, percent homology, presence or absence of certain sequences, for example, restriction sites, percent of codons  
5 changed (such as an increased or decreased usage of certain codons) and expression rates.

As described below, the method was used to create synthetic reporter genes encoding *Renilla reniformis* luciferase, and two click beetle luciferases (one emitting green light and the other emitting red light). For both systems, the  
10 synthetic genes support much greater levels of expression than the corresponding native or parent genes for the protein. In addition, the native and parent genes demonstrated anomalous transcription characteristics when expressed in mammalian cells, which were not evident in the synthetic genes. In particular, basal expression of the native or parent genes is relatively high. Furthermore,  
15 the expression is induced to very high levels by an enhancer sequence in the absence of known promoters. The synthetic genes show lower basal expression and do not show the anomalous enhancer behavior. Presumably, the enhancer is activating transcriptional elements found in the native genes that are absent in the synthetic genes. The results clearly show that the synthetic nucleic acid  
20 sequences exhibit superior performance as reporter genes.

#### Exemplary Uses of the Molecules of the Invention

The synthetic genes of the invention preferably encode the same proteins as their native counterpart (or nearly so), but have improved codon usage while  
25 being largely devoid of known transcription regulatory elements in the coding region. (It is recognized that a small number of amino acid changes may be desired to enhance a property of the native counterpart protein, e.g. to enhance luminescence of a luciferase.) This increases the level of expression of the protein the synthetic gene encodes and reduces the risk of anomalous expression  
30 of the protein. For example, studies of many important events of gene regulation, which may be mediated by weak promoters, are limited by insufficient reporter signals from inadequate expression of the reporter proteins.

The synthetic luciferase genes described herein permit detection of weak promoter activity because of the large increase in level of expression, which enables increased detection sensitivity. Also, the use of some selectable markers may be limited by the expression of that marker in an exogenous cell. Thus, synthetic selectable marker genes which have improved codon usage for that cell, and have a decrease in other undesirable sequences, (e.g., transcription factor binding sites), can permit the use of those markers in cells that otherwise were undesirable as hosts for those markers.

Promoter crosstalk is another concern when a co-reporter gene is used to normalize transfection efficiencies. With the enhanced expression of synthetic genes, the amount of DNA containing strong promoters can be reduced, or DNA containing weaker promoters can be employed, to drive the expression of the co-reporter. In addition, there may be a reduction in the background expression from the synthetic reporter genes of the invention. This characteristic makes synthetic reporter genes more desirable by minimizing the sporadic expression from the genes and reducing the interference resulting from other regulatory pathways.

The use of reporter genes in imaging systems, which can be used for *in vivo* biological studies or drug screening, is another use for the synthetic genes of the invention. Due to their increased level of expression, the protein encoded by a synthetic gene is more readily detectable by an imaging system. In fact, using a synthetic *Renilla* luciferase gene, luminescence in transfected CHO cells was detected visually without the aid of instrumentation.

In addition, the synthetic genes may be used to express fusion proteins, for example fusions with secretion leader sequences or cellular localization sequences, to study transcription in difficult-to-transfect cells such as primary cells, and/or to improve the analysis of regulatory pathways and genetic elements. Other uses include, but are not limited to, the detection of rare events that require extreme sensitivity (e.g., studying RNA recoding), use with IRES, to improve the efficiency of *in vitro* translation or *in vitro* transcription-translation coupled systems such as TNT (Promega Corp., Madison, WI), study of reporters optimized to different host organisms (e.g., plants, fungus, and the like), use of

multiple genes as co-reporters to monitor drug toxicity, as reporter molecules in multiwell assays, and as reporter molecules in drug screening with the advantage of minimizing possible interference of reporter signal by different signal transduction pathways and other regulatory mechanisms.

5           Additionally, uses for the nucleic acid molecules of the invention include fluorescence activated cell sorting (FACS), fluorescent microscopy, to detect and/or measure the level of gene expression *in vitro* and *in vivo*, (e.g., to determine promoter strength), subcellular localization or targeting (fusion protein), as a marker, in calibration, in a kit, (e.g., for dual assays), for *in vivo*  
10   imaging, to analyze regulatory pathways and genetic elements, and in multi-well formats.

          With respect to synthetic DNA encoding luciferases, the use of synthetic click beetle luciferases provides advantages such as the measurement of dual reporters. As *Renilla* luciferase is better suited for *in vivo* imaging (because it  
15   does not depend on ATP or  $Mg^{2+}$  for reaction, unlike firefly luciferase, and because coelenterazine is more permeable to the cell membrane than luciferin), the synthetic *Renilla* luciferase gene can be employed *in vivo*. Further, the synthetic *Renilla* luciferase has improved fidelity and sensitivity in dual luciferase assays, e.g., for biological analysis or in drug screening platform.

20

#### Demonstration of the Invention Using Luciferase Genes

          The reporter genes for click beetle luciferase and *Renilla* luciferase were used to demonstrate the invention because the reaction catalyzed by the protein they encode are significantly easier to quantify than the product of most genes.  
25   However, for the purposes of demonstrating the present invention they represent genes in general.

          Although the click beetle luciferase and *Renilla* luciferase genes share the name "luciferase", this should not be interpreted to mean that they originate from the same family of genes. The two luciferase proteins are evolutionarily  
30   distinct; they have fundamentally different traits and physical structures, they use vastly different substrates (Figure 17), and they evolved from completely different families of genes. The click beetle luciferase is 61 kD in size, uses

luciferin as a substrate and evolved from the CoA synthetases. The *Renilla* luciferase originates from the sea pansy *Renilla Reniformis*, is 35 kD in size, uses coelenterazine as a substrate and evolved from the  $\alpha\beta$  hydrolases. The only shared trait of these two enzymes is that the reaction they catalyze results in light output. They are no more similar for resulting in light output than any other two enzymes would be, for example, simply because the reaction they catalyze results in heat.

Bioluminescence is the light produced in certain organisms as a result of luciferase-mediated oxidation reactions. The luciferase genes, e.g., the genes from luminous beetles, sea pansy, and, in particular, the luciferase from *Photinus pyralis* (the common firefly of North America), are currently the most popular luminescent reporter genes. Reference is made to Bronstein et al. (1994) for a review of luminescent reporter gene assays and to Wood (1995) for a review of the evolution of beetle bioluminescence. See Figure 17 for an illustration of the reactions catalyzed by each of firefly and click beetle luciferases (17A) and *Renilla* luciferase (17B).

Firefly luciferase and *Renilla* luciferase are highly valuable as genetic reporters due to the convenience, sensitivity and linear range of the luminescence assay. Today, luciferase is used in virtually every type of experimental biological system, including, but not limited to, prokaryotic and eukaryotic cell culture, transgenic plants and animals, and cell-free expression systems. The firefly luciferase enzyme is derived from a specific North American beetle, *Photinus pyralis*. The firefly luciferase enzyme and the click beetle luciferase enzyme are monomeric proteins (61 kDa) which generate light through monooxygenation of beetle luciferin utilizing ATP and O<sub>2</sub> (Figure 17A). The *Renilla* luciferase is derived from the sea pansy *Renilla reniformis*. The *Renilla* luciferase enzyme is a 36 kDa monomeric protein that utilizes O<sub>2</sub> and coelenterazine to generate light (Figure 17B).

The gene encoding firefly luciferase was cloned from *Photinus pyralis*, and demonstrated to produce active enzyme in *E. coli* (de Wet et al., 1987). The cDNA encoding firefly luciferase (*luc*) continues to gain favor as the gene of choice for reporting genetic activity in animal, plant and microbial cells. The

firefly luciferase reaction, modified by the addition of CoA to produce persistent light emission, provides an extremely sensitive and rapid *in vitro* assay for quantifying firefly luciferase expression in small samples of transfected cells or tissues.

5           To use firefly luciferase or click beetle luciferase as a genetic reporter, extracts of cells expressing the luciferase are mixed with substrates (beetle luciferin,  $Mg^{2+}$  ATP, and  $O_2$ ), and luminescence is measured immediately. The assay is very rapid and sensitive, providing gene expression data with little effort. The conventional firefly luciferase assay has been further improved by  
10   including coenzyme A in the assay reagent to yield greater enzyme turnover and thus greater luminescence intensity (Promega Luciferase Assay Reagent, Cat.# El500, Promega Corporation, Madison, Wis.). Using this reagent, luciferase activity can be readily measured in luminometers or scintillation counters. Firefly and click beetle luciferase activity can also be detected in living cells in  
15   culture by adding luciferin to the growth medium. This *in situ* luminescence relies on the ability of beetle luciferin to diffuse through cellular and peroxisomal membranes and on the intracellular availability of ATP and  $O_2$  in the cytosol and peroxisome.

          Further, although reporter genes are widely used to measure transcription  
20   events, their utility can be limited by the fidelity and efficiency of reporter expression. For example, in U.S. Patent No. 5,670,356, a firefly luciferase gene (referred to as luc+) was modified to improve the level of luciferase expression. While a higher level of expression was observed, it was not determined that higher expression had improved regulatory control.

25           The invention will be further described by the following nonlimiting examples.

### Example 1

#### Synthetic Click Beetle (RD and GR) Luciferase Nucleic Acid Molecules

30           LucPpLYG is a wild-type click beetle luciferase that emits yellow-green luminescence (Wood, 1989). A mutant of LucPpLYG named YG#81-6G01 was envisioned. YG#81-6G01 lacks a peroxisome targeting signal, has a lower  $K_M$

for luciferin and ATP, has increased signal stability and increased temperature stability when compared to the wild type (PCT/WO9914336). YG #81-6G01 was mutated to emit green luminescence by changing Ala at position 224 to Val (A224V is a green-shifting mutation), or to emit red luminescence by simultaneously introducing the amino acid substitutions A224H, S247H, N346I, and H348Q (red-shifting mutation set) (PCT/WO9518853)

Using YG #81-6G01 as a parent gene, two synthetic gene sequences were designed. One codes for a luciferase emitting green luminescence (GR) and one for a luciferase emitting red luminescence (RD). Both genes were designed to 1) have optimized codon usage for expression in mammalian cells, 2) have a reduced number of transcriptional regulatory sites including mammalian transcription factor binding sites, splice sites, poly(A) addition sites and promoters, as well as prokaryotic (*E. coli*) regulatory sites, 3) be devoid of unwanted restriction sites, e.g., those which are likely to interfere with standard cloning procedures, and 4) have a low DNA sequence identity compared to each other in order to minimize genetic rearrangements when both are present inside the same cell. In addition, desired sequences, e.g., a Kozak sequence or restriction enzyme recognition sites, may be identified and introduced.

Not all design criteria could be met equally well at the same time. The following priority was established for reduction of transcriptional regulatory sites: elimination of transcription factor (TF) binding sites received the highest priority, followed by elimination of splice sites and poly(A) addition sites, and finally prokaryotic regulatory sites. When removing regulatory sites, the strategy was to work from the lesser important to the most important to ensure that the most important changes were made last. Then the sequence was rechecked for the appearance of new lower priority sites and additional changes made as needed. Thus, the process for designing the synthetic GR and RD gene sequences, using computer programs described herein, involved 5 optionally iterative steps that are detailed below

1. Optimized codon usage and changed A224V to create GRver1, separately changed A224H, S247H, H348Q and N346I to create



RDver1. These particular amino acid changes were maintained throughout all subsequent manipulations to the sequence.

2. Removed undesired restriction sites, prokaryotic regulatory sites, splice

5 sites, poly(A) sites thereby creating GRver2 and RDver2.

3. Removed transcription factor binding sites (first pass) and removed any

newly created undesired sites as listed in step 2 above thereby creating

10 GRver3 and RDver3.

4. Removed transcription factor binding sites created by step 3 above (second pass) and removed any newly created undesired sites as listed in step 2 above thereby creating GRver4 and RDver4.

5. Removed transcription factor binding sites created by step 4 above (third

15 Pass) and confirmed absence of sites listed in step 2 above thereby creating GRver5 and RDver5.

6. Constructed the actual genes by PCR using synthetic oligonucleotides corresponding to fragments of GRver5 and RDver5 designed

20 sequences (Figures 6 and 10) thereby creating GR6 and RD7. GR6, upon sequencing was found to have the serine residue at amino acid position 49 mutated to an asparagine and the proline at amino acid position 230 mutated to a serine (S49N, P230S). RD7, upon

25 sequencing was found to have the histidine at amino acid position 36 mutated to a tyrosine (H36Y). These changes occurred during the PCR process.

7. The mutations described in step 6 above (S49N, P230S for GR6 and H36Y for RD7) were reversed to create GRver5.1 and RDver5.1.

30 8. RDver5.1 was further modified by changing the arginine codon at position 351 to a glycine codon (R351G) thereby creating RDver5.2 with improved spectral properties compared to RDver5.1.

9. RDver5.2 was further mutated to increase luminescence intensity thereby creating RD156-1H9 which encodes four additional amino acid changes (M2I, S349T, K488T, E538V) and three silent single base changes (SEQ ID NO:18).

5

1. Optimize codon usage and introduce mutations determining luminescence color

The starting gene sequence for this design step was YG #81-6G01 (SEQ ID NO:2).

10 **a) Optimize codon usage:**

The strategy was to adapt the codon usage for optimal expression in human cells and at the same time to avoid *E. coli* low-usage codons. Based on these requirements, the best two codons for expression in human cells for all amino acids with more than two codons were selected (see Wada et al., 1990).

- 15 In the selection of codon pairs for amino acids with six codons, the selection was biased towards pairs that have the largest number of mismatched bases to allow design of GR and RD genes with minimum sequence identity (codon distinction):

|    |              |              |              |
|----|--------------|--------------|--------------|
|    | Arg: CGC/CGT | Leu: CTG/TTG | Ser: TCT/AGC |
| 20 | Thr: ACC/ACT | Pro: CCA/CCT | Ala: GCC/GCT |
|    | Gly: GGC/GGT | Val: GTC/GTG | Ile: ATC/ATT |

- Based on this selection of codons, two gene sequences encoding the YG#81-6G01 luciferase protein sequence were computer generated. The two genes were designed to have minimum DNA sequence identity and at the same time closely similar codon usage. To achieve this, each codon in the two genes was replaced by a codon from the limited list described above in an alternating fashion (e.g., Arg<sub>(n)</sub> is CGC in gene 1 and CGT in gene 2, Arg<sub>(n+1)</sub> is CGT in gene 1 and CGC in gene 2).
- 25

- For subsequent steps in the design process it was anticipated that changes had to be made to this limited optimal codon selection in order to meet other design criteria, however, the following low-usage codons in mammalian cells were not used unless needed to meet criteria of higher priority:
- 30

Arg: CGA    Leu: CTA    Ser: TCG  
 Pro: CCG    Val: GTA    Ile: ATA

Also, the following low-usage codons in *E. coli* were avoided when reasonable  
 (note that 3 of these match the low-usage list for mammalian cells):

5        Arg: CGA/CGG/AGA/AGG  
          Leu: CTA    Pro: CCC    Ile: ATA

**b) Introduce mutations determining luminescence color:**

10        Into one of the two codon-optimized gene sequences was introduced the  
          single green-shifting mutation and into the other were introduced the 4 red-  
          shifting mutations as described above.

         The two output sequences from this first design step were named GRver1  
 (version 1 GR) and RDver1 (version 1 RD). Their DNA sequences are 63%  
 identical (594 mismatches), while the proteins they encode differ only by the 4  
 15        amino acids that determine luminescence color (see Figures 2 and 3 for an  
          alignment of the DNA and protein sequences).

         Tables 1 and 2 show, as an example, the codon usage for valine and  
 leucine in human genes, the parent gene YG#81-6G01, the codon-optimized  
 synthetic genes GRver1 and RDver1, as well as the final versions of the  
 20        synthetic genes after completion of step 5 in the design process (GRver5 and  
          RDver5). For a complete summary of the codon changes, see Figures 4 and 5.

Table 1: Valine

| Codon | Human | Parent | GR ver1 | RD ver1 | GR ver5 | RD ver5 |
|-------|-------|--------|---------|---------|---------|---------|
| GTA   | 4     | 13     | 0       | 0       | 1       | 1       |
| GTC   | 13    | 4      | 25      | 24      | 21      | 26      |
| GTG   | 24    | 12     | 25      | 25      | 25      | 17      |
| GTT   | 9     | 20     | 0       | 0       | 3       | 5       |

Table 2: Leucine

| Codon | Human | Parent | GR ver1 | RD ver1 | GR ver5 | RD ver5 |
|-------|-------|--------|---------|---------|---------|---------|
| CTA   | 3     | 5      | 0       | 0       | 0       | 0       |
| CTC   | 12    | 4      | 0       | 1       | 12      | 11      |
| CTG   | 24    | 4      | 28      | 27      | 19      | 18      |

|     |   |    |    |    |    |    |
|-----|---|----|----|----|----|----|
| CTT | 6 | 12 | 0  | 0  | 1  | 1  |
| TTA | 3 | 17 | 0  | 0  | 0  | 0  |
| TTG | 6 | 13 | 27 | 27 | 23 | 25 |

2. Remove undesired restriction sites, prokaryotic regulatory sites, splice sites and poly(A) addition sites

The starting gene sequences for this design step were GRver1 and RDver1.

5    **a) Remove undesired restriction sites:**

To check for the presence and location of undesired restriction sites, the sequences of both synthetic genes were compared against a database of restriction enzyme recognition sequences (REBASE ver.712,

<http://www.neb.com/rebase>) using standard sequence analysis software

10    (GenePro ver 6.10, Riverside Scientific Ent.).

Specifically, the following restriction enzymes were classified as undesired:

- *Bam*H I, *Xho* I, *Sfi* I, *Kpn* I, *Sac* I, *Mlu* I, *Nhe* I, *Sma* I, *Xho* I, *Bgl* II, *Hind* III, *Nco* I, *Nar* I, *Xba* I, *Hpa* I, *Sal* I,
- other cloning sites commonly used: *Eco*R I, *Eco*R V, *Cla* I,
- 15    - eight-base cutters (commonly used for complex constructs),
- *Bst*E II (to allow N-terminal fusions),
- *Xcm* I (can generate A/T overhang used for T-vector cloning).

To eliminate undesired restriction sites when found in a synthetic gene, one or more codons of the synthetic gene sequence were altered in accordance with the codon optimization guidelines described in 1a above.

20

**b) Remove prokaryotic (*E. coli*) regulatory sequences:**

To check for the presence and location of prokaryotic regulatory sequences, the sequences of both synthetic genes were searched for the presence of the following consensus sequences using standard sequence analysis software

25    (GenePro):

- TATAAT (-10 Pribnow box of promoter)
- AGGA or GGAG (ribosome binding site; only considered if paired with a methionine codon 12 or fewer bases downstream).

To eliminate such regulatory sequences when found in a synthetic gene, one or more codons of the synthetic gene at sequence were altered in accordance with the codon optimization guidelines described in 1a above.

**c) Remove splice sites:**

5 To check for the presence and location of splice sites, the DNA strand corresponding to the primary RNA transcript of each synthetic gene was searched for the presence of the following consensus sequences (see Watson et al., 1983) using standard sequence analysis software (GenePro):

- 10 - splice donor site: AG | GTRAGT (exon | intron), the search was performed for AGGTRAG and the lower stringency GGTRAGT;
- splice acceptor site: (Y)<sub>n</sub>NCAG | G (intron | exon), the search was performed with n = 1.

To eliminate splice sites found in a synthetic gene, one or more codons of the synthetic gene sequence were altered in accordance with the codon optimization  
15 guidelines described in 1a above. Splice acceptor sites were generally difficult to eliminate in one gene without introducing them into the other gene because they tended to contain one of the two only Gln codons (CAG); they were removed by placing the Gln codon CAA in both genes at the expense of a slightly increased sequence identity between the two genes.

20 **d) Remove poly(A) addition sites:**

To check for the presence and location of poly(A) addition sites, the sequences of both synthetic genes were searched for the presence of the following consensus sequence using standard sequence analysis software (GenePro):

- 25 - AATAAA.

To eliminate each poly(A) addition site found in a synthetic gene, one or more codons of the synthetic gene sequence were altered in accordance with the codon optimization guidelines described in 1a above. The two output sequences from this second design step were named GRver2 and RDver2. Their DNA sequences  
30 are 63% identical (590 mismatches) (Figs. 2 and 3).

3. Remove transcription factor (TF) binding sites, then repeat steps 2 a-d

The starting gene sequences for this design step were GRver2 and RDver2.

To check for the presence, location and identity of potential TF binding sites, the sequences of both synthetic genes were used as query sequences to search a database of transcription factor binding sites (TRANSFAC v3.2). The TRANSFAC database (<http://transfac.gbf.de/TRANSFAC/index.html>) holds information on gene regulatory DNA sequences (TF binding sites) and proteins (TFs) that bind to and act through them. The SITE table of TRANSFAC Release 3.2 contains 4,401 entries of individual (putative) TF binding sites (including TF binding sites in eukaryotic genes, in artificial sequences resulting from mutagenesis studies and *in vitro* selection procedures based on random oligonucleotide mixtures or specific theoretical considerations, and consensus binding sequences (from Faisst and Meyer, 1992)).

The software tool used to locate and display these TF binding sites in the synthetic gene sequences was TESS (Transcription Element Search Software, <http://agave.humgen.upenn.edu/tess/index.html>). The filtered string-based search option was used with the following user-defined search parameters:

- Factor Selection Attribute: Organism Classification
- Search Pattern: Mammalia
- Max. Allowable Mismatch %: 0
- Min. element length: 5
- Min. log-likelihood: 10

This parameter selection specifies that only mammalian TF binding sites (approximately 1,400 of the 4,401 entries in the database) that are at least 5 bases long will be included in the search. It further specifies that only TF binding sites that have a perfect match in the query sequence and a minimum log likelihood (LLH) score of 10 will be reported. The LLH scoring method assigns 2 to an unambiguous match, 1 to a partially ambiguous match (e.g., A or T match W) and 0 to a match against 'N'. For example, a search with parameters specified above would result in a "hit" (positive result or match) for TATAA (SEQ ID NO:240) (LLH = 10), STRATG (SEQ ID NO:241) (LLH = 10), and MTTNCNNMA (SEQ ID NO:242) (LLH = 10) but not for TRATG (SEQ ID

NO: 243) (LLH = 9) if these four TF binding sites were present in the query sequence. A lower stringency test was performed at the end of the design process to re-evaluate the search parameters.

When TESS was tested with a mock query sequence containing known  
5 TF binding sites it was found that the program was unable to report matches to sites ending with the 3' end of the query sequence. Thus, an extra nucleotide was added to the 3' end of all query sequences to eliminate this problem.

The first search for TF binding sites using the parameters described above found about 100 transcription factor binding sites (hits) for each of the  
10 two synthetic genes (GRver2 and RDver2). All sites were eliminated by changing one or more codons of the synthetic gene sequences in accordance with the codon optimization guidelines described in 1a above. However, it was expected that some these changes created new TF binding sites, other regulatory sites, and new restriction sites. Thus, steps 2 a-d were repeated as described, and  
15 4 new restriction sites and 2 new splice sites were removed. The two output sequences from this third design step were named GRver3 and RDver3. Their DNA sequences are 66% identical (541 mismatches) (Figs. 2 and 3).

#### 4. Remove new transcription factor (TF) binding sites, then repeat steps 2 a-d

20 The starting gene sequences for this design step were GRver3 and RDver3.

This fourth step is an iteration of the process described in step 3. The search for newly introduced TF binding sites yielded about 50 hits for each of the two synthetic genes. All sites were eliminated by changing one or more codons of  
25 the synthetic gene sequences in general accordance with the codon optimization guidelines described in 1a above. However, more high to medium usage codons were used to allow elimination of all TF binding sites. The lowest priority was placed on maintaining low sequence identity between the GR and RD genes. Then steps 2 a-d were repeated as described. The two output sequences from  
30 this fourth design step were named GRver4 and RDver4. Their DNA sequences are 68% identical (506 mismatches) (Figs 2 and 3).

5. Remove new transcription factor (TF) binding sites, then repeat steps 2 a-d

The starting gene sequences for this design step were GRver4 and RDver4.

This fifth step is another iteration of the process described in step 3 above. The search for new TF binding sites introduced in step 4 yielded about 20 hits for each of the two synthetic genes. All sites were eliminated by changing one or more codons of the synthetic gene sequences in general accordance with the codon optimization guidelines described in 1a above. However, more high to medium usage codons were used (these are all considered "preferred") to allow elimination of all TF binding sites. The lowest priority was placed on maintaining low sequence identity between the GR and RD genes. Then steps 2 a-d were repeated as described. Only one acceptor splice site could not be eliminated. As a final step the absence of all TF binding sites in both genes as specified in step 3 was confirmed. The two output sequences from this fifth and last design step were named GRver5 and RDver5. Their DNA sequences are 69% identical (504 mismatches) (Figs. 2 and 3).

Additional evaluation of GRver5 and RDver5

**a) Use lower stringency parameters for TESS:**

- The search for TF binding sites was repeated as described in step 3 above, but with even less stringent user-defined parameters:
- setting LLH to 9 instead of 10 did not result in new hits;
  - setting LLH to 0 through 8 (incl.) resulted in hits for two additional sites, MAMAG (22 hits) and CTKTK (24 hits);
  - setting LLH to 8 and the minimum element length to 4, the search yielded (in addition to the two sites above) different 4-base sites for AP-1, NF-1, and c-Myb that are shortened versions of their longer respective consensus sites which were eliminated in steps 3-5 above.

It was not realistic to attempt complete elimination of these sites without introduction of new sites, so no further changes were made.

**b) Search different database:**



The Eukaryotic Promoter Database (release 45) contains information about reliably mapped transcription start sites (1253 sequences) of eukaryotic genes. This database was searched using BLASTN 1.4.11 with default parameters (optimized to find nearly identical sequences rapidly; see Altschul et al, 1990) at the National Center for Biotechnology Information site (<http://www.ncbi.nlm.nih.gov/cgi-bin/BLAST>). To test this approach, a portion of pGL3-Control vector sequence containing the SV40 promoter and enhancer was used as a query sequence, yielding the expected hits to SV40 sequences. No hits were found when using the two synthetic genes as query sequences.

#### Summary of GRver5 and RDver5 synthetic gene properties

Both genes, which at this stage were still only "virtual" sequences in the computer, have a codon usage that strongly favors mammalian high-usage codons and minimizes mammalian and *E. coli* low-usage codons. Figure 4 shows a summary of the codon usage of the parent gene and the various synthetic gene versions.

Both genes are also completely devoid of eukaryotic TF binding sites consisting of more than four unambiguous bases, donor and acceptor splice sites (one exception: GRver5 contains one splice acceptor site), poly(A) addition sites, specific prokaryotic (*E. coli*) regulatory sequences, and undesired restriction sites.

The gene sequence identity between GRver5 and RDver5 is only 69% (504 base mismatches) while their encoded proteins are 99% identical (4 amino acid mismatches), see Figures 2 and 3. Their identity with the parent sequence YG#81-6G1 is 74% (GRver5) and 73% (RDver5), see Figure 2. Their base composition is 49.9% GC (GRver5) and 49.5% GC (RDver5), compared to 40.2% GC for the parent YG#81-6G01.

#### Construction of synthetic genes

The two synthetic genes were constructed by assembly from synthetic oligonucleotides in a thermocycler followed by PCR amplification of the full-length genes (similar to Stemmer et al. (1995) *Gene*, 164, pp. 49-53).

Unintended mutations that interfered with the design goals of the synthetic genes were corrected.

**a) Design of synthetic oligonucleotides:**

5           The synthetic oligonucleotides were mostly 40mers that collectively code for both complete strands of each designed gene (1,626 bp) plus flanking regions needed for cloning (1,950 bp total for each gene; Figure 6). The 5' and 3' boundaries of all oligonucleotides specifying one strand were generally placed in a manner to give an average offset/overlap of 20 bases relative to the boundaries  
10 of the oligonucleotides specifying the opposite strand.

          The ends of the flanking regions of both genes matched the ends of the amplification primers (pRAMtailup: 5'-gtactgagacgacgccagcccaagcttaggcctgagtg SEQ ID NO:229, and pRAMtaildn: 5'-ggcatgagcgtgaactgactgaactagcggccgccgag SEQ ID NO:230) to allow cloning of the genes into our *E. coli* expression vector  
15 pRAM (WO99/14336).

          A total of 183 oligonucleotides were designed (Figure 6): fifteen oligonucleotides that collectively encode the upstream and downstream flanking sequences (identical for both genes; SEQ ID NOs: 35-49) and 168 oligonucleotides (4 x 42) that encode both strands of the two genes (SEQ ID  
20 NOs: 50-217).

          All 183 oligonucleotides were run through the hairpin analysis of the OLIGO software (OLIGO 4.0 Primer Analysis Software © 1989-1991 by Wojciech Rychlik) to identify potentially detrimental intra-molecular loop formation. The guidelines for evaluating the analysis results were set according  
25 to recommendations of Dr. Sims (Sigma-Genosys Custom Gene Synthesis Department): oligos forming hairpins with  $\Delta G < -10$  have to be avoided, those forming hairpins with  $\Delta G \leq -7$  involving the 3' end of the oligonucleotide should also be avoided, while those with an overall  $\Delta G \leq -5$  should not pose a problem for this application. The analysis identified 23 oligonucleotides able to form  
30 hairpins with a  $\Delta G$  between -7.1 and -4.9. Of these, 5 had blocked or nearly blocked 3' ends (0-3 free bases) and were re-designed by removing 1-4 bases at their 3' end and adding it to the adjacent oligonucleotide.

The 40mer oligonucleotide covering the sequence complementary to the poly(A) tail had a very low complexity 3' end (13 consecutive T bases). An additional 40mer was designed with a high complexity 3' end but a consequently reduced overlap with one of its complementary oligonucleotides (11 instead of 20 bases) on the opposite strand.

Even though the oligos were designed for use in a thermocycler-based assembly reaction, they could also be used in a ligation-based protocol for gene construction. In this approach, the oligonucleotides are annealed in a pairwise fashion and the resulting short double-stranded fragments are ligated using the sticky overhangs. However, this would require that all oligonucleotides be phosphorylated.

#### b) Gene assembly and amplification

In a first step, each of the two synthetic genes was assembled in a separate reaction from 98 oligonucleotides. The total volume for each reaction was 50  $\mu$ l:

0.5  $\mu$ M oligonucleotides (= 0.25 pmoles of each oligo)

1.0 U *Taq* DNA polymerase

0.02 U *Pfu* DNA polymerase

20 2 mM  $MgCl_2$

0.2 mM dNTPs (each)

0.1% gelatin

Cycling conditions: (94°C for 30 seconds, 52°C for 30 seconds, and 72°C for 30 seconds) x 55 cycles.

25 In a second step, each assembled synthetic gene was amplified in a separate reaction. The total volume for each reaction was 50  $\mu$ l:

2.5 l assembly reaction

5.0 U *Taq* DNA polymerase

0.1 U *Pfu* DNA polymerase

30 1 M each primer (pRAMtailup, pRAMtaildn)

2 mM  $MgCl_2$

0.2 mM dNTPs (each)

Cycling conditions: (94°C for 20 seconds, 65°C for 60 seconds, 72°C for 3 minutes) x 30 cycles.

The assembled and amplified genes were subcloned into the pRAM vector and expressed in *E. coli*, yielding 1-2% luminescent GR or RD clones.

- 5 Five GR and five RD clones were isolated and analyzed further. Of the five GR clones, three had the correct insert size, of which one was weakly luminescent and one had an altered restriction pattern. Of the five RD clones, two had the correct size insert with an altered restriction pattern and one of those was weakly luminescent. Overall, the analysis indicated the presence of a large number of mutations in the genes, most likely the result of errors introduced in the assembly and amplification reactions.
- 10

#### c) Corrective assembly and amplification

- To remove the large number of mutations present in the full-length synthetic genes we performed an additional assembly and amplification reaction for each gene using the proof-reading DNA polymerase *Tli*. The assembly reaction contained, in addition to the 98 GR or RD oligonucleotides, a small amount of DNA from the corresponding full-length clones with mutations described above. This allows the oligos to correct mutations present in the templates.
- 15
- 20

The following assembly reaction was performed for each of the synthetic genes. The total volume for each reaction was 50 µl:

- 0.5 µM oligonucleotides (= 0.25 pmoles of each oligo)  
0.016 pmol plasmid (mix of clones with correct insert size)  
25 2.5 U *Tli* DNA polymerase  
2 mM MgCl<sub>2</sub>  
0.2 mM dNTPs (each)  
0.1% gelatin  
30 Cycling conditions: 94°C for 30 seconds, then (94°C for 30 seconds, 52°C for 30 seconds, 72°C for 30 seconds) for 55 cycles, then 72°C for 5 minutes.

The following amplification reaction was performed on each of the assembly reactions. The total volume for each amplification reaction was 50  $\mu$ l:

- 1-5  $\mu$ l of assembly reaction
- 40 pmol each primer (pRAMtailup, pRAMtaildn)
- 5 2.5 U *Tli* DNA polymerase
- 2 mM MgCl<sub>2</sub>
- 0.2 mM dNTPs (each)
- Cycling conditions: 94°C for 30 seconds, then (94°C for 20 seconds, 65°C for 60 seconds and 72°C for 3 minutes)
- 10 for 30 cycles, then 72°C for 5 minutes.

The genes obtained from the corrective assembly and amplification step were subcloned into the pRAM vector and expressed in *E. coli*, yielding 75% luminescent GR or RD clones. Forty-four GR and 44 RD clones were analyzed with our screening robot (WO99/14336). The six best GR and RD clones were manually analyzed and one best GR and RD clone was selected (GR6 and RD7). Sequence analysis of GR6 revealed two point mutations in the coding region, both of which resulted in an amino acid substitution (S49N and P230S). Sequence analysis of RD7 revealed three point mutations in the coding region, one of which resulted in an amino acid substitution (H36Y). It was confirmed that none of the silent point mutations introduced any regulatory or restriction sites conflicting with the overall design criteria for the synthetic genes.

#### d) Reversal of unintended amino acid substitutions

The unintended amino acid substitutions present in the GR6 and RD7 synthetic genes were reversed by site-directed mutagenesis to match the GRver5 and RDver5 designed sequences, thereby creating GRver5.1 and RDver5.1. The DNA sequences of the mutated regions were confirmed by sequence analysis.

#### e) Improve spectral properties

The RDver5.1 gene was further modified to improve its spectral properties by introducing an amino change (R351G), thereby creating RDver5.2



The cycling parameters were: 94°C for 5 minutes; (94°C for 30 seconds; 55°C for 1 minute; and 72°C for 3 minutes) x 15 cycles. The purified PCR product was digested with *Nco* I and *Xba* I, ligated with pGL3-control that was also digested with *Nco* I and *Xba* I, and the ligated products introduced to *E. coli*.

- 5 To insert the luciferase genes into the other pGL3 reporter vectors (basic, promoter and enhancer), the pGL3-control vectors containing each of the luciferase genes was digested with *Nco* I and *Xba* I, ligated with other pGL3 vectors that also were digested with *Nco* I and *Xba* I, and the ligated products introduced to *E. coli*. Note that the polypeptide encoded by GRver5.1 and
- 10 RDver5.1 (and RD156-1H9, see below) nucleic acid sequences in pGL3 vectors has an amino acid substitution at position 2 to valine as a result of the *Nco* I site at the initiation codon in the oligonucleotide.

- Because of internal *Nco* I and *Xba* I sites, the native gene in YG #81-6G01 was amplified from a *Hind* III site upstream to a *Hpa* I site downstream of
- 15 the coding region and which included flanking sequences found in the GR and RD clones. The upstream primer (5'-CAA AAA GCT TGG CAT TCC GGT ACT GTT GGT AAA GCC ACC ATG GTG AAG CGA GAG- 3'; SEQ ID NO:234) and a downstream primer (5'- CAA TTG TTG TTG TTA ACT TGT TTA TT -3'; SEQ ID NO:235) were mixed with YG#81-6G01 and amplified
- 20 using the PCR conditions above. The purified PCR product was digested with *Nco* I and *Xba* I, ligated with pGL3-control that was also digested with *Hind* III and *Hpa* I, and the ligated products introduced into *E. coli*. To insert YG#81-6G01 into the other pGL3 reporter vectors (basic, promoter and enhancer), the pGL3-control vectors containing YG#81-6G01 were digested with *Nco* I and
- 25 *Xba* I, ligated with the other pGL3 vectors that also were digested with *Nco* I and *Xba* I, and the ligated products introduced to *E. coli*. Note that the clone of YG#81-6G01 in the pGL3 vectors has a C instead of an A at base 786, which yields a change in the amino acid sequence at residue 262 from Phe to Leu (Figure 2 shows the sequence of YG#81-6G01 prior to introduction into pGL3
- 30 vectors). To determine whether the altered amino acid at position 262 affected the enzyme biochemistry, the clone of YG#81-6G01 was mutated to resemble the original sequence. Both clones were then tested for expression in *E. coli*,

physical stability, substrate binding, and luminescence output kinetics. No significant differences were found.

Partially purified enzymes expressed from the synthetic genes and the parent gene were employed to determine  $K_M$  for luciferin and ATP (see Table 3).

Table 3

| Enzyme    | $K_M$ (LH <sub>2</sub> ) | $K_M$ (ATP) |
|-----------|--------------------------|-------------|
| YG parent | 2 $\mu$ M                | 17 $\mu$ M  |
| GR        | 1.3 $\mu$ M              | 25 $\mu$ M  |
| RD        | 24.5 $\mu$ M             | 46 $\mu$ M  |

*In vitro* eukaryotic transcription/translation reactions were also conducted using Promega's TNT T7 Quick system according to manufacturer's instructions. Luminescence levels were 1 to 37-fold and 1 to 77-fold higher (depending on the reaction time) for the synthetic GR and RD genes, respectively, compared to the parent gene (corrected for luminometer spectral sensitivity).

To test whether the synthetic click beetle luciferase genes and the wild type click beetle gene have improved expression in mammalian cells, each of the synthetic genes and the parent gene was cloned into a series of pGL3 vectors and introduced into CHO cells (Table 8). In all cases, the synthetic click beetle genes exhibited a higher expression than the native gene. Specifically, expression of the synthetic GR and RD genes was 1900-fold and 40-fold higher, respectively, than that of the parent (transfection efficiency normalized by comparison to native *Renilla* luciferase gene). Moreover, the data (basic versus control vector) show that the synthetic genes have reduced basal level transcription.

Further, in experiments with the enhancer vector where the percentage of activity in reference to the control is compared between the native and synthetic gene, the data showed that the synthetic genes have reduced risk of anomalous transcription characteristics. In particular, the parent gene appeared to contain one or more internal transcriptional regulatory sequences that are activated by



the enhancer in the vector, and thus is not suitable as a reporter gene while the synthetic GR and RD genes showed a clean reporter response (transfection efficiency normalized by comparison to native *Renilla* luciferase gene). See

Table 9.

- 5 The clone names and their corresponding SEQ ID numbers for nucleotide sequence and amino acid sequence are listed below in Table 4.

Table 4

|    | Clone name | Luciferase Type              | SEQ ID NO. | SEQ ID NO. |
|----|------------|------------------------------|------------|------------|
| 10 | LUCPPLYG   | Wild type YG Click Beetle    | 1          | 23         |
|    | YG#81-6G01 | Mutant YG Click Beetle       | 2          | 24         |
|    | GRver1     | Synthetic Green Click Beetle | 3          | 25         |
|    | GRver2     | Synthetic Green Click Beetle | 4          | 26         |
| 15 | GRver3     | Synthetic Green Click Beetle | 5          | 27         |
|    | GRver4     | Synthetic Green Click Beetle | 6          | 28         |
|    | GRver5     | Synthetic Green Click Beetle | 7          | 29         |
|    | GR6        | Synthetic Green Click Beetle | 8          | 30         |
|    | GRver5.1   | Synthetic Green Click Beetle | 9          | 31         |
| 20 | RDver1     | Synthetic Red Click Beetle   | 10         | 32         |
|    | RDver2     | Synthetic Red Click Beetle   | 11         | 33         |
|    | RDver3     | Synthetic Red Click Beetle   | 12         | 34         |
|    | RDver4     | Synthetic Red Click Beetle   | 13         | 218        |
|    | RDver5     | Synthetic Red Click Beetle   | 14         | 219        |
| 25 | RD7        | Synthetic Red Click Beetle   | 15         | 220        |
|    | RDver5.1   | Synthetic Red Click Beetle   | 16         | 221        |
|    | RDver5.2   | Synthetic Red Click Beetle   | 17         | 222        |
|    | RD156-1H9  | Synthetic Red Click Beetle   | 18         | 223        |
| 30 | RELLUC     | Wild type <i>Renilla</i>     | 19         | 224        |
|    | Rlucver1   | Synthetic <i>Renilla</i>     | 20         | 225        |
|    | Rlucver2   | Synthetic <i>Renilla</i>     | 21         | 226        |

Rluc-final

Synthetic *Renilla*

22

227

### Example 2

5

#### Evolution of the RD luciferase gene

RDver5.2 was mutated to increase its luminescence intensity, thereby creating RD156-1H9 which carries four additional amino acid changes (M2I, S349T, K488T, E538V) and three silent point mutations (SEQ ID NO:18).

#### **a) Site-directed mutagenesis:**

10

The initial strategy was to use site-directed mutagenesis. There are four amino acid differences between the GR and RD synthetic genes with H348Q providing the greatest contribution to red color. Thus, this substitution may also cause structural changes in the protein that could lead to low light output. Optimization of positions near this area could increase light output. The

15

following positions were selected for mutagenesis:

1. S344 (at the edge of the binding pocket for luciferin) – randomize this codon.
2. A245 (strictly conserved but closest to 348 and at the edge of the active site pocket) – randomize this codon.
- 20 3. I347 (not conserved, next to 348 in sequence) – mutate to hydrophobic amino acids only.
4. S349 (not conserved, next to 348 in sequence) – mutate to S, T, A, P only.

Oligonucleotides designed to mutate the above positions were used in a  
25 site-directed mutagenesis experiment (WO99/14336) and the resulting mutants were screened for luminescence intensity. There was little variation in light intensity and only about 25% were luminescent. For more detailed analysis, clones were picked and analyzed with the screening robot (PCT/WO9914336). None of the clones had a luminescence intensity (LI) higher than RDver5.2, but  
30 four of the clones had slightly lower composite  $K_m$  for luciferin and ATP ( $K_m$ ).

#### **b) Directed evolution:**

Protocols and procedures used for the directed evolution are detailed in see PCT/WO9914336. DNA from the four clones with lower  $K_m$  was combined and three libraries of random mutants were produced. The libraries were screened with the robot and clones with the highest LI values were selected.

- 5 These clones were shuffled together and another robotic screen was completed with an incubation temperature of 46°C. The three clones with the highest LI values were RD156-0B4, RD156-1A5, and RD156-1H9.

**c) Analysis:**

- The three clones with the highest LI values were selected for manual analysis to  
10 confirm that their luminescence intensity was higher than that of RDver5.2 and to ensure that their spectral properties were not compromised. One of the clones was slightly green-shifted, all others maintained the spectral properties of RDver5.2 (Table 5).

Table 5

| Clone              | Peak (nm) | Width (nm) |
|--------------------|-----------|------------|
| RD156-0B4          | 616       | 68         |
| RD156-1A5          | 614       | 70         |
| RD156-1H9          | 618       | 69         |
| Rdver5.2 (prep #1) | 617       | 70         |
| Rdver5.2 (prep #2) | 618       | 69         |

15

- The  $K_m$  values for luciferin and the luminescence intensity relative to RDver5.2 were determined for all three clones in several independent experiments. All cells samples were processed with CCLR lysis buffer (E1483, Promega Corp., Madison, WI) and diluted 1: 10 into buffer (25 mM HEPES pH  
20 7.8, 5% glycerol, 1 mg/ml BSA, 150 mM NaCl). Table 7 summarizes the results (Lum: luminescence values were normalized to optical density; measurements for independent experiments are separated by forward slashes) from expression in bacterial cells. RD156-1H9, the clone with the highest luminescence intensity (5 to 10-fold increase) also has an about 2-fold higher  $K_m$  for luciferin.

25

Table 6

| Clone | $K_m$ Luciferin [ $\mu$ M] | Lum (normalized to RDver5.2) |
|-------|----------------------------|------------------------------|
|-------|----------------------------|------------------------------|

|                    |              |                |
|--------------------|--------------|----------------|
| RD156-0B4          | 8 / 10       | 2.2 / 2.5      |
| RD156-1A5          | 13 / 13      | 3.1 / 5.6      |
| RD156-1H9          | 20 / 23 / 23 | 4 / 10.9 / 7.5 |
| RDver5.2 (prep #1) | 12 / 14 / 14 |                |
| RDver5.2 (prep #2) | 40 / 50      |                |
| GRver5.1 (prep #1) | 0.5          | 64             |
| GRver5.1 (prep #2) | 3            |                |

Table 7 shows a comparison between the luminescence intensities of RD156-1H9, GRver5.1 and RDver5.2 normalized to GRver5.1 with and without correction for the spectral sensitivity of the luminometer photomultiplier tube.

- 5 With correction, the luminescence intensity of clone RD156-1H9 was only about 2-fold lower than that of GRver5.1. The luciferin Km for clone RD156-1H9 is approximately 40-fold higher than GRver5.1. RD156-1H9 is thermostable at 50°C for at least 2 hours.

10

Table 7

| Name      | No Correction | With Correction |
|-----------|---------------|-----------------|
| RDver5.2  | 0.016         | 0.06            |
| GRver5.1  | 1.000         | 1.00            |
| RD156-1H9 | 0.116         | 0.45            |

- 15 Tables 8 and 9 show a comparison of luciferase expression levels in CHO cells. Table 8 shows the expression levels only from the control vectors in comparison to the firefly luciferase gene (RLU = relative light units). Table 9 shows a comparison of the expression levels in all four pGL3 vectors calculated as a percent of the expression level in pGL3-control.

20

Table 8Synthetic Click Beetle Gene Expression

|                       |            |
|-----------------------|------------|
| <u>Control vector</u> | <u>rlu</u> |
| YG#81-6G01            | 177        |

| <u>Control vector</u> | <u>rlu</u> |
|-----------------------|------------|
| GRver5.1              | 343,417    |
| RDver5.1              | 7,161      |
| RD156-1H9             | 20,802     |
| FireFly               | 488,016    |

Table 9Synthetic Click Beetle Gene Expression

| <u>Vector</u>      | <u>Percent of control<br/>vector</u> |
|--------------------|--------------------------------------|
| YG-control         | 100                                  |
| RD-control         | 100                                  |
| GR-control         | 100                                  |
| RD156-1H9 control  | 100                                  |
| YG-basic           | 3.3                                  |
| RD-basic           | 1.0                                  |
| GR-basic           | 0.2                                  |
| RD156-1H9 basic    | 0.3                                  |
| YG-promoter        | 4.2                                  |
| RD-promoter        | 15.1                                 |
| GR-promoter        | 5.7                                  |
| RD156-1H9 promoter | 15.5                                 |
| YG-enhancer        | 51.5                                 |
| RD-enhancer        | 2.8                                  |
| GR-enhancer        | 1.4                                  |
| RD156-1H9 enhancer | 0.3                                  |

5

Example 3Synthetic *Renilla* Luciferase Nucleic Acid Molecule

The synthetic *Renilla* luciferase genes prepared include 1) an introduced  
10 Kozak sequence, 2) codon usage optimized for mammalian (human) expression,  
3) a reduction or elimination of unwanted restriction sites, 4) removal of  
prokaryotic regulatory sites (ribosome binding site and TATA box), 5) removal  
of splice sites and poly(A) addition sites, and 6) a reduction or elimination of  
mammalian transcriptional factor binding sequences.

15 The process of computer-assisted design of synthetic *Renilla* luciferase  
genes by iterative rounds of codon optimization and removal of transcription

factor binding sites and other regulatory sites as well as restriction sites can be described in three steps:

1. Using the wild type *Renilla* luciferase gene as the parent gene, codon usage was optimized, one amino acid was changed (T→A) to generate a Kozak consensus sequence, and undesired restriction sites were eliminated thereby creating synthetic gene Rlucver1.
2. Remove prokaryotic regulatory sites, splice sites, poly(A) sites and transcription factor (TF) binding sites (first pass). Then remove newly created TF binding sites. Then remove newly created undesired restriction enzyme sites, prokaryotic regulatory sites, splice sites, and poly(A) sites without introducing new TF binding sites. This thereby created Rlucver2.
3. Change 3 bases of Rlucver2 thereby creating Rluc-final.
4. The actual gene was then constructed from synthetic oligonucleotides corresponding to the Rluc-final designed sequence. All mutations resulting from the assembly or PCR process were corrected. This gene is Rluc-final (SEQ ID NO:22) and encodes the amino acid sequence of SEQ ID NO:227.

#### Codon Selection

Starting with the *Renilla reniformis* luciferase sequence in Genbank (Accession No. M63501, SEQ ID NO:19), codons were selected based on codon usage for optimal expression in human cells and to avoid *E. coli* low-usage codons. The best codon for expression in human cells (or the best two codons if found at a similar frequency) was chosen for all amino acids with more than one codon (Wada et al., 1990):

|    |              |          |
|----|--------------|----------|
| 25 | Arg: CGC     | Lys: AAG |
|    | Leu: CTG     | Asn: AAC |
|    | Ser: TCT/AGC | Gln: CAG |
|    | Thr: ACC     | His: CAC |
|    | Pro: CCA/CCT | Glu: GAG |
| 30 | Ala: GCC     | Asp: GAC |
|    | Gly: GGC     | Tyr: TAC |
|    | Val: GTG     | Cys: TGC |

Ile: ATC/ATT      Phe: TTC

In cases where two codons were selected for one amino acid, they were used in an alternating fashion. To meet other criteria for the synthetic gene, the initial optimal codon selection was modified to some extent later. For example, introduction of a Kozak sequence required the use of GCT for Ala at amino acid position 2 (see below).

The following low-usage codons in mammalian cells were not used unless needed: Arg: CGA, CGU; Leu: CTA, UUA; Ser: TCG; Pro: CCG; Val: GTA; and Ile: ATA. The following low-usage codons in *E. coli* were also avoided when reasonable (note that 3 of these match the low-usage list for mammalian cells): Arg: CGA/CGG/AGA/AGG, Leu: CTA; Pro: CCC; Ile: ATA.

#### Introduction of Kozak Sequences

The Kozak sequence: 5' aaccATGGCT 3' (SEQ ID NO: 293) (the *Nco* I site is underlined, the coding region is shown in capital letters) was introduced to the synthetic *Renilla* luciferase gene. The introduction of the Kozak sequence changes the second amino acid from Thr to Ala (GCT).

#### Removal of undesired restriction sites

REBASE ver. 808 (updated August 1, 1998; Restriction Enzyme Database; [www.neb.com/rebase](http://www.neb.com/rebase)) was employed to identify undesirable restriction sites as described in Example 1. The following undesired restriction sites (in addition to those described in Example 1) were removed according to the process described in Example 1: *Eco*ICR I, *Nde*I, *Nsi*I, *Sph*I, *Spe*I, *Xma*I, *Pst*I.

The version of *Renilla* luciferase (Rluc) which incorporates all these changes is Rlucver1.

#### Removal of prokaryotic (*E. coli*) regulatory sequences, splice sites, and poly(A) sites

The priority and process for eliminating transcription regulation sites was as described in Example 1.

#### Removal of TF binding sites

The same process, tools, and criteria were used as described in Example 1, however, the newer version 3.3 of the TRANSFAC database was employed.

After removing prokaryotic regulatory sequences, splice sites and poly(A) sites from Rlucver1, the first search for TF binding sites identified about 60 hits. All sites were eliminated with the exception of three that could not be removed without altering the amino acid sequence of the synthetic *Renilla* gene:

1. site at position 63 composed of two codons for W (TGGTGG), for CAC-binding protein T00076;
2. site at position 522 composed of codons for KMV (AAN ATG GTN), for myc-DF1 T00517;
3. site at position 885 composed of codons for EMG (GAR ATG GGN), for myc-DF1 T00517.

The subsequent second search for (newly introduced) TF binding sites yielded about 20 hits. All new sites were eliminated, leaving only the three sites described above. Finally, any newly introduced restriction sites, prokaryotic regulatory sequences, splice sites and poly(A) sites were removed without introducing new TF binding sites if possible.

Rlucver2 was obtained (SEQ ID Nos. 21 and 226).

As in Example 1, lower stringency search parameters were specified for the TESS filtered string search to further evaluate the synthetic *Renilla* gene.

With the LLH reduced from 10 to 9 and the minimum element length reduced from 5 to 4, the TESS filtered string search did not show any new hits. When, in addition to the parameter changes listed above, the organism classification was expanded from "mammalia" to "chordata", the search yielded only four more TF binding sites. When the Min LLH was further reduced to between 8 and 0, the search showed two additional 5-base sites (MAMAG and CTKTK) which combined had four matches in Rlucver2, as well as several 4-base sites. Also as in Example 1, Rlucver2 was checked for hits to entries in the EPD (Eukaryotic Promoter Database, Release 45). Three hits were determined (one to *Mus musculus* promoter H-2L<sup>d</sup> (Cell, 44, 261 (1986), one to Herpes Simplex Virus type 1 promoter b'g'2.7 kb, and one to *Homo sapiens* DHFR



promoter (J. Mol. Biol., 176, 169 (1984)). However, no further changes were made to Rlucver2.

---

#### Summary of Properties for Rlucver2

- 5     -     All 30 low usage codons were eliminated. The introduction of a Kozak sequence changed the second amino acid from Thr to Ala;
- base composition: 55.7% GC (*Renilla* wild-type parent gene: 36.5%);
- one undesired restriction site could not be eliminated: *EcoR* V at position 488;
- 10    -     the synthetic gene had no prokaryotic promoter sequence but one potentially functional ribosome binding site (RBS) at positions 867-73 (about 13 bases upstream of a Met codon ) could not be eliminated;
- all poly(A) addition sites were eliminated;
- splice sites: 2 donor splice sites could not be eliminated (both share the amino acid sequence MGK);
- 15    -     TF sites: all sites with a consensus of >4 unambiguous bases were eliminated (about 280 TF binding sites were removed) with 3 exceptions due to the preference to avoid changes to the amino acid sequence.

Synthetic *Renilla* luciferase sequences are shown in Figures 7 and 8. A codon  
20    usage comparison is shown in Figure 9.

When introduced into pGL3, Rluc-final has a Kozak sequence (CACCATGGCT). The changes in Rluc-final relative to Rlucver2 were introduced during gene assembly. One change was at position 619, a C to an A, which eliminated a eukaryotic promoter sequence and reduced the stability of a  
25    hairpin structure in the corresponding oligonucleotide employed to assemble the gene. Other changes included a change from CGC to AGA at positions 218-220 (resulted in a better oligonucleotide for PCR).

#### Gene Assembly Strategy

30       The gene assembly protocol employed for the synthetic *Renilla* luciferase was similar to that described in Example 1. The oligonucleotides employed are shown in Figure 10.

Sense Strand primer:

5' AACCATGGCTTCCAAGGTGTAAGACCCGGAGCAACGCAAA-3' (SEQ ID NO:236)

5 Anti-sense Strand primer:

5' GCTCTAGAATTACTGCTCGTTCTTCAGCACGCGCTCCACG 3' (SEQ ID NO:237)

The resulting synthetic gene fragment was cloned into a pRAM vector using *Nco* I and *Xba* I. Two clones having the correct size insert were sequenced. Four to six mutations were found in the synthetic gene from each clone. These mutations were fixed by site-directed mutagenesis (Gene Editor from Promega Corp., Madison, WI) and swapping the correct regions between these two genes. The corrected gene was confirmed by sequencing.

#### 15 Other Vectors

To prepare an expression vector for the synthetic *Renilla* luciferase gene in a pGL-3 control vector backbone, 5 µg of pGL3-control was digested with *Nco* I and *Xba* I in 50 µl final volume with 2 µl of each enzyme and 5 µl 10X buffer B (nanopure water was used to fill the volume to 50 µl). The digestion reaction was incubated at 37°C for 2 hours, and the whole mixture was run on a 1% agarose gel in 1XTAE. The desired vector backbone fragment was purified using Qiagen's QIAquick gel extraction kit.

The native *Renilla* luciferase gene fragment was cloned into pGL3-control vector using two oligonucleotides, *Nco* I-RL-F and *Xba* I-RL-R, to PCR amplify native *Renilla* luciferase gene using pRL-CMV as the template. The sequence for *Nco* I-RL-F is 5'-CGCTAGCCATGGCTTCGAAAGTTTATGATCC-3' (SEQ ID NO:238); the sequence for *Xba* I-RL-R is 5' GGCCAGTAACTCTAGAATTATTGTT-3' (SEQ ID NO:239). The PCR reaction was carried out as follows:

Reaction mixture (for 100 µl):

|                        |                          |
|------------------------|--------------------------|
| DNA template (Plasmid) | 1.0 µl (1.0 ng/µl final) |
|------------------------|--------------------------|

|    |                           |  |
|----|---------------------------|--|
|    | 10 X Rec. Buffer          | 10.0 $\mu$ l (Stratagene Corp.)                |
| 5  | dNTPs (25 mM each)        | 1.0 $\mu$ l (final 250 $\mu$ M)                |
|    | Primer 1 (10 $\mu$ M)     | 2.0 $\mu$ l (0.2 $\mu$ M final)                |
|    | Primer 2 (10 $\mu$ M)     | 2.0 $\mu$ l (0.2 $\mu$ M final)                |
| 10 | <i>Pfu</i> DNA Polymerase | 2.0 $\mu$ l (2.5 U/ $\mu$ l, Stratagene Corp.) |

82.0  $\mu$ l double distilled water

PCR Reaction: heat 94°C for 2 minutes; (94°C for 20 seconds;  
65°C for 1 minute; 72°C for 2 minutes; then 72°C for 5 minutes) x 25 cycles,  
15 then incubate on ice. The PCR amplified fragment was cut from a gel, and the  
DNA purified and stored at -20°C.

To introduce native *Renilla* luciferase gene fragment into pGL3-control  
vector, 5  $\mu$ g of the PCR product of the native *Renilla* luciferase gene (RAM-RL-  
synthetic) was digested with *Nco* I and *Xba* I. The desired *Renilla* luciferase  
20 gene fragment was purified and stored at -20°C.

Then 100 ng of insert and 100 ng of pGL3-control vector backbone were  
digested with restriction enzymes *Nco* I and *Xba* I and ligated together. Then 2  
 $\mu$ l of the ligation mixture was transformed into JM109 competent cells. Eight  
ampicillin resistance clones were picked and their DNA isolated. DNA from  
25 each positive clone of pGL3-control-native and pGL3-control-synthetic was  
purified. The correct sequences for the native gene and the synthetic gene in the  
vectors were confirmed by DNA sequencing.

To determine whether the synthetic *Renilla* luciferase gene has improved  
expression in mammalian cells, the gene was cloned into the mammalian  
30 expression vector pGL3-control vector under the control of SV40 promoter and  
SV40 early enhancer (Fig. 13A). The native *Renilla* luciferase gene was also  
cloned into the pGL-3 control vector so that the expression from synthetic gene  
and the native gene could be compared. The expression vectors were then  
transfected into four common mammalian cell lines (CHO, NIH3T3, HeLa and  
35 CV-1; Table 10), and the expression levels compared between the vectors with  
the synthetic gene versus the native gene. The amount of DNA used was at two

different levels to ascertain that expression from the synthetic gene is consistently increased at different expression levels. The results show a 70-600 fold increase of expression for the synthetic *Renilla* luciferase gene in these cells (Table 10).

5

Table 10

Enhanced Synthetic *Renilla* Gene Expression

| <u>Cell Type</u> | <u>Amount Vector</u> | <u>Fold Expression Increase</u> |
|------------------|----------------------|---------------------------------|
| CHO              | 0.2 µg               | 142                             |
|                  | 2.8 µg               | 145                             |
| NIH3T3           | 0.2 µg               | 326                             |
|                  | 2.0 µg               | 593                             |
| HeLa             | 0.2 µg               | 185                             |
|                  | 1.0 µg               | 103                             |
| CV-1             | 0.2 µg               | 68                              |
|                  | 2.0 µg               | 72                              |

10 One important advantage of luciferase reporter is its short protein half-life. The enhanced expression could also result from extended protein half-life and, if so, this gives an undesired disadvantage of the new gene. This possibility is ruled out by a cycloheximide chase ("CHX Chase") experiment (Figure 14), which demonstrated that there was no increase of protein half-life resulted from  
 15 the humanized *Renilla* luciferase gene.

To ensure that the increase in expression is not limited to one expression vector backbone, is promoter specific and/or cell specific, a synthetic *Renilla* gene (Rluc-final) as well as native *Renilla* gene were cloned into different vector backbones and under different promoters (Figure 13B). The synthetic gene  
 20 always exhibited increased expression compared to its wild-type counterpart (Table 11).

25

Table 11

Renilla Gene Expression: native v. synthetic (Rluc-final)

| <u>Vector</u>               | NIH-3T3   | HeLa      | CHO       |
|-----------------------------|-----------|-----------|-----------|
| pRL-tk, native              | 3,834.6   | 922.4     | 7,671.9   |
| pRL-tk, synthetic           | 13,252.5  | 9,040.2   | 41,743.5  |
| pRL-CMV, native             | 168,062.2 | 842,482.5 | 153,539.5 |
| pRL-CMV, synthetic          | 2,168,129 | 8,440,306 | 2,532,576 |
| pRL-SV40, native            | 224,224.4 | 346,787.6 | 85,323.6  |
| pRL-SV40, synthetic         | 1,469,588 | 2,632,510 | 1,422,830 |
| pRL-null, native            | 2,853.8   | 431.7     | 2,434     |
| pRL-null, synthetic         | 9,151.17  | 2,439     | 28,317.1  |
| pRGL3b, native              | 12        | 21.8      | 17        |
| pRGL3b, synthetic           | 130.5     | 212.4     | 1,094.5   |
| pRGL3-tk, native            | 27.9      | 155.5     | 186.4     |
| pRGL3-tk, synthetic         | 6,778.2   | 8,782.5   | 9,685.9   |
| pRL-tk no intron, native    | 31.8      | 165       | 93.4      |
| pRL-tk no intron, synthetic | 6,665.5   | 6,379     | 21,433.1  |

Table 12

Renilla Luciferase Expression in Mammalian Cells

5

Percent of control vector

| <u>Vector</u>          | <u>CHO cells</u> | <u>NIH3T3 cells</u> | <u>HeLa cells</u> |
|------------------------|------------------|---------------------|-------------------|
| pRL-control native     | 100              | 100                 | 100               |
| pRL-control synthetic  | 100              | 100                 | 100               |
| pRL-basic native       | 4.1              | 5.6                 | 0.2               |
| pRL-basic synthetic    | 0.4              | 0.1                 | 0.0               |
| pRL-promoter native    | 5.9              | 7.8                 | 0.6               |
| pRL-promoter synthetic | 15.0             | 9.9                 | 1.1               |

|                        | <u>Percent of control vector</u> |       |      |
|------------------------|----------------------------------|-------|------|
| pRL-enhancer native    | 42.1                             | 123.9 | 52.7 |
| pRL-enhancer synthetic | 2.6                              | 1.5   | 5.4  |

(Vector backbones illustrated in Figure 13A)

With reduced spurious expression the synthetic gene should exhibit less basal level transcription in a promoterless vector. The synthetic and native *Renilla* luciferase genes were cloned into the pGL3-basic vector to compare the basal level of transcription. Because the synthetic gene itself has increased expression efficiency, the activity from the promoterless vector cannot be compared directly to judge the difference in basal transcription, rather, this is taken into consideration by comparing the percentage of activity from the promoterless vector in reference to the control vector (expression from the basic vector divided by the expression in the fully functional expression vector with both promoter and enhancer elements). The data demonstrate that the synthetic *Renilla* luciferase has a lower level of basal transcription than the native gene (Table 12)

It is well known to those skilled in the art that an enhancer can substantially stimulate promoter activity. To test whether the synthetic gene has reduced risk of inappropriate transcriptional characteristics, the native and synthetic gene were introduced into a vector with an enhancer element (pGL3-enhancer vector). Because the synthetic gene has higher expression efficiency, the activity of both cannot be compared directly to compare the level of transcription in the presence of the enhancer, however, this is taken into account by using the percentage of activity from enhancer vector in reference to the control vector (expression in the presence of enhancer divided by the expression in the fully functional expression vector with both promoter and enhancer elements). Such results show that when native gene is present, the enhancer alone is able to stimulate transcription from 42-124% of the control, however, when the native gene is replaced by the synthetic gene in the same vector, the activity only constitutes 1-5% of the value when the same enhancer and a strong

SV40 promoter are employed. This clearly demonstrates that synthetic gene has reduced risk of spurious expression (Table 12).

The synthetic *Renilla* gene (Rluc-final) was used in *in vitro* systems to compare translation efficiency with the native gene. In a T7 quick coupled transcription/translation system (Promega Corp., Madison, WI), pRL-null native plasmid (having the native *Renilla* luciferase gene under the control of the T7 promoter) or the same amount of pRL-null-synthetic plasmid (having the synthetic *Renilla* luciferase gene under the control of the T7 promoter) was added to the TNT reaction mixture and luciferase activity measured every 5 minutes up to 60 minutes. Dual Luciferase assay kit (Promega Corp.) was used to measure *Renilla* luciferase activity. The data showed that improved expression was obtained from the synthetic gene (Figure 15A,B). To further evidence the increased translation efficiency of the synthetic gene, RNA was prepared by an *in vitro* transcription system, then purified. pRL-null (native or synthetic) vectors were linearized with *Bam*H I. The DNA was purified by multiple phenol-chloroform extraction followed by ethanol precipitation. An *in vitro* T7 transcription system was employed by prepare RNAs. The DNA template was removed by using RNase-free DNase, and RNA was purified by phenol-chloroform extraction followed by multiple isopropanol precipitations. The same amount of purified RNA, either for the synthetic gene or the native gene, was then added to a rabbit reticulocyte lysate (Figure 15 C, D) or wheat germ lysate (Figure 15 E, F). Again, the synthetic *Renilla* luciferase gene RNA produced more luciferase than the native one. These data suggest that the translation efficiency is improved by the synthetic sequence. To determine why the synthetic gene was highly expressed in wheat germ, plant codon usage was determined. The lowest usage codons in higher plants coincided with those in mammals.

Reporter gene assays are widely used to study transcriptional regulation events. This is often carried out in co-transfection experiments, in which, along with the primary reporter construct containing the testing promoter, a second control reporter under a constitutive promoter is transfected into cells as an internal control to normalize experimental variations including transfection

efficiencies between the samples. Control reporter signal, potential promoter cross talk between the control reporter and primary reporter, as well as potential regulation of the control reporter by experimental conditions, are important aspects to consider for selecting a reliable co-reporter vector.

5 As described above, vector constructs were made by cloning synthetic *Renilla* luciferase gene into different vector backbones under different promoters. All the constructs showed higher expression in the three mammalian cell lines tested (Table 11). Thus, with better expression efficiency, the synthetic *Renilla* luciferase gives out higher signal when transfected into mammalian cells.

10 Because a higher signal is obtained, less promoter activity is required to achieve the same reporter signal, this reduced risk of promoter interference. CHO cells were transfected with 50 ng pGL3-control (firefly *luc+*) plus one of 5 different amounts of native pRL-TK plasmid (50, 100, 500, 1000, or 2000 ng) or synthetic pRL-TK (5, 10, 50, 100, or 200 ng). To each transfection, pUC19  
15 carrier DNA was added to a total of 3 µg DNA. Shown in Figure 16 is the experiment demonstrating that 10 fold less pRL-TK DNA gives similar or more signal as the native gene, with reduced risk of inhibiting expression from the primary reporter pGL3-control.

Experimental treatment sometimes may activate cryptic sites within the  
20 gene and cause induction or suppression of the co-reporter expression, which would compromise its function as co-reporter for normalization of transfection efficiencies. One example is that TPA induces expression of co-reporter vectors harboring the wild-type gene when transfecting MCF-7 cells. 500 ng pRL-TK (native), 5 µg native and synthetic pRG-B, 2.5 µg native and synthetic pRG-TK  
25 were transfected per well of MCF-7 cells. 100 ng/well pGL3-control (firefly *luc+*) was co-transfected with all RL plasmids. Carrier DNA, pUC19, was used to bring the total DNA transfected to 5.1 µg/well. 15.3 µl TransFast Transfection Reagent (Promega Corp., Madison, WI) was added per well. Sixteen hours later, cells were trypsinized, pooled and split into six wells of a 6-well dish and  
30 allowed to attach to the well for 8 hours. Three wells were then treated with the 0.2 nM of the tumor promoter, TPA (phorbol-12-myristate-13-acetate, Calbiochem #524400-S), and three wells were mock treated with 20 µl DMSO.



Cells were harvested with 0.4 ml Passive Lysis Buffer 24 hours post TPA addition. The results showed that by using the synthetic gene, undesirable change of co-reporter expression by experimental stimuli can be avoided (Table 13). This demonstrates that using synthetic gene can reduce the risk of anomalous expression.

Table 13  
TPA Induction

| <u>Vector</u>               | <u>Rlu</u> | <u>Fold Induction</u> |
|-----------------------------|------------|-----------------------|
| pRL-tk untreated (native)   | 184        |                       |
| pRL-tk TPA treated (native) | 812        | 4.4                   |
| pRG-B untreated (native)    | 1          |                       |
| pRG-B TPA treated (native)  | 8          | 8.0                   |
| pRG-B untreated (final)     | 132        |                       |
| pRG-B TPA treated (final)   | 195        | 1.47                  |
| pRG-tk untreated (native)   | 44         |                       |
| pRG-tk TPA treated (native) | 192        | 4.36                  |
| pRG-tk untreated (final)    | 12,816     |                       |
| pRG-tk TPA treated (final)  | 11,347     | 0.88                  |

#### References

- 10 Altschul et al., Nucl. Acids Res., 25, 3389 (1997).
- Aota et al., Nucl. Acids Res., 16, 315 (1988).
- Boshart et al., Cell, 41, 521 (1985).
- Bronstein et al., Cal. Biochem., 219, 169 (1994).
- Corpet et al., Nucl. Acids Res., 16, 881 (1988).
- 15 deWet et al., Mol. Cell. Biol., 7, 725 (1987).
- Dijkema et al., EMBO J., 4, 761 (1985).
- Faist and Meyer, Nucl. Acids Res., 20, 26 (1992).
- Gorman et al., Proc. Natl. Acad. Sci. USA, 79, 6777 (1982).
- Higgins et al., Gene, 73, 237 (1985).

- Higgins et al., CABIOS, 5, 151 (1989).  
Huang et al., CABIOS, 8, 155 (1992).  
----- Itolcik et al., PNAS, 94, 12410 (1997).  
Johnson et al., Mol. Reprod. Devel., 50, 377 (1998).  
5 Jones et al., Mol. Cell. Biol., 17, 6970 (1997).  
Karlín and Altschul, Proc. Natl. Acad. Sci. USA, 87, 2264 (1990).  
Karlín and Altschul, Proc. Natl. Acad. Sci. USA, 90, 5873 (1993).  
Keller et al., J. Cell Biol., 84, 3264 (1987).  
Kim et al., Gene, 91, 217 (1990).  
10 Lamb et al., Mol. Reprod. Devel., 51, 218 (1998).  
Mariatis et al., Science, 236, 1237 (1987).  
Michael et al., EMBO. J., 9, 481 (1990).  
Mizushima and Nagata, Nucl. Acids Res., 18, 5322 (1990).  
Murray et al., Nucl. Acids Res., 17, 477 (1989).  
15 Myers and Miller, CABIOS, 4, 11 (1988).  
Needleman and Wunsen, J. Mol. Biol., 48, 443 (1970).  
Pearson and Lipman, Proc. Natl. Acad. Sci. USA, 85, 2444 (1988).  
Pearson et al., Meth. Mol. Biol., 24, 307 (1994).  
Sharp et al., Nucl. Acids Res., 16, 8207 (1988).  
20 Sharp et al., Nucl. Acids Res., 15, 1281 (1987).  
Smith and Waterman, Adv. Appl. Math., 2, 482 (1981).  
Stemmer et al., Gene, 164, 49 (1995).  
Uetsuki et al., J. Biol. Chem., 264, 5791 (1989).  
Voss et al., Trends Biochem. Sci., 11, 287 (1986).  
25 Wada et al., Nucl. Acids Res., 18, 2367 (1990).  
Watson et al, eds. Recombinant DNA: A Short Course, Scientific American  
Books, W. H. Freeman and Company, New York (1983).  
Wood, K. Photochemistry and Photobiology, 62, 662 (1995).  
Wood, K. Science 244, 700 (1989)

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All publications, patents and patent applications are incorporated herein by reference. While in the foregoing specification, this invention has been

described in relation to certain preferred embodiments thereof, and many details have been set forth for purposes of illustration, it will be apparent to those skilled in the art that the invention is susceptible to additional embodiments and that

5 certain of the details herein may be varied considerably without departing from the basic principles of the invention.

**WHAT IS CLAIMED IS:**

1. --- A synthetic nucleic acid molecule comprising at least 300 nucleotides of a coding region for a polypeptide, having a codon composition differing at more than 25% of the codons from a wild type nucleic acid sequence encoding a polypeptide, and having at least 3-fold fewer transcription regulatory sequences relative to the average number of such sequences resulting from random selections of codons at the codons which differ, wherein the transcription regulatory sequences are selected from the group consisting of transcription factor binding sequences, intron splice sites, poly(A) addition sites and promoter sequences, and wherein the polypeptide encoded by the synthetic nucleic acid molecule has at least 85% sequence identity to the polypeptide encoded by the wild type nucleic acid sequence.
2. The synthetic nucleic acid molecule of claim 1 wherein the synthetic nucleic acid molecule has at least 5-fold fewer transcription regulatory sequences.
3. The synthetic nucleic acid molecule of claim 1 wherein the codon composition of the synthetic nucleic acid molecule differs from the wild type nucleic acid sequence at more than 35% of the codons.
4. The synthetic nucleic acid molecule of claim 1 wherein the codon composition of the synthetic nucleic acid molecule differs from the wild type nucleic acid sequence at more than 45% of the codons.
5. The synthetic nucleic acid molecule of claim 1 wherein the codon composition of the synthetic nucleic acid molecule differs from the wild type nucleic acid sequence at more than 55% of the codons.

6. The synthetic nucleic acid molecule of claim 1 wherein the majority of codons which differ are ones that are preferred codons of a desired host cell.

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7. The synthetic nucleic acid molecule of claim 1 wherein the synthetic nucleic acid molecule encodes a reporter molecule.
8. The synthetic nucleic acid molecule of claim 1 wherein the synthetic nucleic acid molecule encodes a selectable marker protein.
9. The synthetic nucleic acid molecule of claim 1 wherein the synthetic nucleic acid molecule encodes a luciferase.
10. The synthetic nucleic acid molecule of claim 9 wherein the wild type nucleic acid sequence encodes a *Renilla* luciferase.
11. The synthetic nucleic acid molecule of claim 9 wherein the wild type nucleic acid sequence encodes a beetle luciferase.
12. The synthetic nucleic acid molecule of claim 11 wherein the synthetic nucleic acid molecule encodes the amino acid valine at position 224.
13. The synthetic nucleic acid molecule of claim 11 wherein the synthetic nucleic acid molecule encodes the amino acid histidine at position 224, histidine at position 247, isoleucine at position 346, glutamine at position 348, or any combination thereof.
14. The synthetic nucleic acid molecule of claim 1 wherein the majority of codons which differ in the synthetic nucleic acid molecule are those which are employed more frequently in mammals.

15. The synthetic nucleic acid molecule of claim 1 wherein the majority of codons which differ in the synthetic nucleic acid molecule are those which are preferred codons in humans.
16. The synthetic nucleic acid molecule of claim 1 wherein the majority of codons which differ in the synthetic nucleic acid molecule are those which are preferred codons in plants.
17. The synthetic nucleic acid molecule of claim 9 wherein the synthetic nucleic acid molecule comprises SEQ ID NO:21 (Rlucver2) or SEQ ID NO:22 (Rluc-final).
18. The synthetic nucleic acid molecule of claim 9 wherein the synthetic nucleic acid molecule comprises SEQ ID NO:7 (GRver5), SEQ ID NO:8 (GRver6), SEQ ID NO:9 (GRver5.1), or SEQ ID NO:297 (GRver5.1).
19. The synthetic nucleic acid molecule of claim 9 wherein the synthetic nucleic acid molecule comprises SEQ ID NO:14 (RDver5), SEQ ID NO:15 (RDver7), SEQ ID NO:16 (RDver5.1), SEQ ID NO:299 (RDver5.1), SEQ ID NO:17 (RDver5.2), SEQ ID NO:18 (RD156-1H9) or SEQ ID NO:301 (RD156-1H9).
20. The synthetic nucleic acid molecule of claim 15 wherein the majority of codons which differ are the human codons CGC, CTG, TCT, AGC, ACC, CCA, CCT, GCC, GGC, GTG, ATC, ATT, AAG, AAC, CAG, CAC, GAG, GAC, TAC, TGC and TTC.
21. The synthetic nucleic acid molecule of claim 15 wherein the majority of codons which differ are the human codons CGC, CTG, TCT, ACC, CCA, GCC, GGC, GTC, and ATC or codons CGT, TTG, AGC, ACT, CCT, GCT, GGT, GTG and ATT.

22. The synthetic nucleic acid molecule of claim 16 wherein the majority of codons which differ are the plant codons CGC, CTT, TCT, TCC, ACC, CCA, CCT, GCT, GGA, GTG, ATC, ATT, AAG, AAC, CAA, CAC, GAG, GAC, TAC, TGC and TTC.
23. The synthetic nucleic acid molecule of claim 16 wherein the majority of codons which differ are the plant codons CGC, CTT, TCT, ACC, CCA, GTC, GGA, GTC, and ATC or codons CGT, TGG, AGC, ACT, CCT, GCC, GGT, GTG and ATT.
24. The synthetic nucleic acid molecule of claim 1 wherein the synthetic nucleic acid molecule is expressed in a mammalian host cell at a level which is greater than that of the wild type nucleic acid sequence.
25. The synthetic nucleic acid molecule of claim 1 wherein the synthetic nucleic acid molecule has an increased number of CTG or TTG leucine-encoding codons.
26. The synthetic nucleic acid molecule of claim 1 wherein the synthetic nucleic acid molecule has an increased number of GTG or GTC valine-encoding codons.
27. The synthetic nucleic acid molecule of claim 1 wherein the synthetic nucleic acid molecule has an increased number of GGC or GGT glycine-encoding codons.
28. The synthetic nucleic acid molecule of claim 1 wherein the synthetic nucleic acid molecule an increased number of ATC or ATT isoleucine-encoding codons.

29. The synthetic nucleic acid molecule of claim 1 wherein the synthetic nucleic acid molecule has an increased number of CCA or CCT proline-encoding codons.
30. The synthetic nucleic acid molecule of claim 1 wherein the synthetic nucleic acid molecule has an increased number of CGC or CGT arginine-encoding codons.
31. The synthetic nucleic acid molecule of claim 1 wherein the synthetic nucleic acid molecule has an increased number of AGC or TCT serine-encoding codons.
32. The synthetic nucleic acid molecule of claim 1 wherein the synthetic nucleic acid molecule has an increased number of ACC or ACT threonine-encoding codons.
33. The synthetic nucleic acid molecule of claim 1 wherein the synthetic nucleic acid molecule has an increased number of GCC or GCT alanine-encoding codons.
34. The synthetic nucleic acid molecule of claim 1 wherein the codons in the synthetic nucleic acid molecule which differ encode the same amino acids as the corresponding codons in the wild type nucleic acid sequence.
35. A plasmid comprising the synthetic nucleic acid molecule of claim 1.
36. An expression vector comprising the synthetic nucleic acid molecule of claim 1 linked to a promoter functional in a cell.
37. The expression vector of claim 36 wherein the synthetic nucleic acid molecule is operatively linked to a Kozak consensus sequence.



38. The expression vector of claim 36 wherein the promoter is functional in a mammalian cell.
39. The expression vector of claim 36 wherein the promoter is functional in a human cell.
40. The expression vector of claim 36 wherein the promoter is functional in a plant cell.
41. The expression vector of claim 36 wherein the expression vector further comprises a multiple cloning site.
42. The expression vector of claim 41 wherein the expression vector comprises a multiple cloning site positioned between the promoter and the synthetic nucleic acid molecule.
43. The expression vector of claim 41 wherein the expression vector comprises a multiple cloning site positioned downstream from the synthetic nucleic acid molecule.
44. A host cell comprising the expression vector of claim 36.
45. A reporter gene expression kit comprising, in suitable container means, the expression vector of claim 36.
46. An isolated polypeptide encoded by SEQ ID NO:9 (GRver5.1) or SEQ ID NO:18 (RD156-1H9).
47. A polynucleotide which hybridizes under stringent hybridization conditions to SEQ ID NO:22 (Rluc-final), SEQ ID NO:9 (GRver5.1), SEQ ID NO:18 (RD156-1H9), SEQ ID NO:297 (GRver5.1), SEQ ID NO:301 (RD156-1H9), or the complement thereof.

48. A method to prepare a synthetic nucleic acid molecule comprising an open reading frame, comprising:
- a) altering a plurality of transcription regulatory sequences in a parent nucleic acid sequence which encodes a polypeptide having at least 100 amino acids to yield a synthetic nucleic acid molecule which has at least 3-fold fewer transcription regulatory sequences relative to the parent nucleic acid sequence, wherein the transcription regulatory sequences are selected from the group consisting of transcription factor binding sequences, intron splice sites, poly(A) addition sites, enhancer sequences and promoter sequences; and
  - b) altering greater than 25% of the codons in the synthetic nucleic acid sequence which has a decreased number of transcription regulatory sequences to yield a further synthetic nucleic acid molecule, wherein the codons which are altered do not result in an increased number of transcription regulatory sequences, wherein the further synthetic nucleic acid molecule encodes a polypeptide with at least 85% amino acid sequence identity to the polypeptide encoded by the parent nucleic acid sequence.
49. A method to prepare a synthetic nucleic acid molecule comprising an open reading frame, comprising:
- a) altering greater than 25% of the codons in a parent nucleic acid sequence which encodes a polypeptide having at least 100 amino acids to yield a codon-altered synthetic nucleic acid molecule, and
  - b) altering a plurality of transcription regulatory sequences in the codon-altered synthetic nucleic acid molecule to yield a further synthetic nucleic acid molecule which has at least 3-fold fewer transcription regulatory sequences relative to a synthetic nucleic acid molecule with a random selection of codons at the codons which differ, wherein the transcription regulatory sequences are selected from the group consisting of transcription factor binding sequences, intron splice sites, poly(A)

addition sites, enhancer sequences and promoter sequences, and wherein the further synthetic nucleic acid molecule encodes a polypeptide with at least 85% amino acid sequence identity to the polypeptide encoded by the parent nucleic acid sequence.

50. The method of claim 48 or 49 wherein the parent nucleic acid sequence encodes a reporter molecule.
51. The method of claim 48 or 49 wherein the parent nucleic acid sequence encodes a luciferase.
52. The method of claim 48 or 49 wherein the synthetic nucleic acid molecule hybridizes under medium stringency hybridization conditions to the parent nucleic acid sequence.
53. The method of claim 48 or 49 wherein the codons which are altered encode the same amino acid as the corresponding codons in the parent nucleic acid sequence.
54. A synthetic nucleic acid molecule which is the further synthetic nucleic acid molecule prepared by the method of claim 48 or 49.
55. A method for preparing at least two synthetic nucleic acid molecules which are codon distinct versions of a parent nucleic acid sequence which encodes a polypeptide, comprising:
  - a) altering a parent nucleic acid sequence to yield a synthetic nucleic acid molecule having an increased number of a first plurality of codons that are employed more frequently in a selected host cell relative to the number of those codons in the parent nucleic acid sequence; and
  - b) altering the parent nucleic acid sequence to yield a further synthetic nucleic acid molecule having an increased number of a second plurality of codons that are employed more frequently in the host cell relative to

the number of those codons in the parent nucleic acid sequence, wherein the first plurality of codons is different than the second plurality of codons, and wherein the synthetic and the further synthetic nucleic acid molecules encode the same polypeptide.

56. The method of claim 55 further comprising altering a plurality of transcription regulatory sequences in the synthetic nucleic acid molecule, the further synthetic nucleic acid molecule, or both, to yield at least one yet further synthetic nucleic acid molecule which has at least 3-fold fewer transcription regulatory sequences relative to the synthetic nucleic acid molecule, the further synthetic nucleic acid molecule, or both.
57. The method of claim 55 further comprising altering at least one codon in the first synthetic sequence to yield a first modified synthetic sequence which encodes a polypeptide with at least one amino acid substitution relative to the polypeptide encoded by the first synthetic nucleic acid sequence.
58. The method of claim 56 further comprising altering at least one codon in the second synthetic sequence to yield a second modified synthetic sequence which encodes a polypeptide with at least one amino acid substitution relative to the polypeptide encoded by the first synthetic nucleic acid sequence.
59. The method of claim 55 wherein the synthetic sequences encode a luciferase.
60. The synthetic nucleic acid molecule of claim 1 wherein the synthetic nucleic acid molecule is expressed at a level which is at least 110% of that of the wild type nucleic acid sequence in a cell or cell extract under identical conditions.

61. The synthetic nucleic acid molecule of claim 1 wherein the polypeptide encoded by the synthetic nucleic acid molecule has at least 90% contiguous sequence identity to the polypeptide encoded by the wild type nucleic acid sequence.
62. The synthetic nucleic acid molecule of claim 1 wherein the polypeptide encoded by the synthetic nucleic acid molecule is identical in amino acid sequence to the polypeptide encoded by the wild type nucleic acid sequence.
63. A vector comprising a synthetic nucleic acid molecule having at least 3-fold fewer transcriptional regulatory sequences relative to a vector comprising a parent nucleic acid sequence, wherein the transcription regulatory sequences are selected from the group consisting of transcription factor binding sequences, intron splice sites, poly(A) addition sites and promoter sequences.
64. The vector of claim 63 wherein the synthetic nucleic acid molecule does not encode a polypeptide.
65. The method of claim 48 or 49 further comprising altering the further synthetic nucleic acid molecule to encode a polypeptide having at least one amino acid substitution relative to the polypeptide encoded by the parent nucleic acid sequence.
66. The method of claim 48 or 49 wherein the altering of transcription regulatory sequences does not introduce amino acid substitutions to the polypeptide encoded by the synthetic nucleic acid molecule.

**Figure 1**  
The Genetic Code

| First<br>Position<br>(5' end) | Second position |     |      |      | Third<br>position<br>(3' end) |
|-------------------------------|-----------------|-----|------|------|-------------------------------|
| U                             | U               | C   | A    | G    |                               |
|                               | Phe             | Ser | Tyr  | Cys  | U                             |
|                               | Phe             | Ser | Tyr  | Cys  | C                             |
|                               | Leu             | Ser | Stop | Stop | A                             |
| C                             | Leu             | Ser | Stop | Trp  | G                             |
|                               | Leu             | Pro | His  | Arg  | U                             |
|                               | Leu             | Pro | His  | Arg  | C                             |
|                               | Leu             | Pro | Gln  | Arg  | A                             |
| A                             | Leu             | Pro | Gln  | Arg  | G                             |
|                               | Ile             | Thr | Asn  | Ser  | U                             |
|                               | Ile             | Thr | Asn  | Ser  | C                             |
|                               | Ile             | Thr | Lys  | Arg  | A                             |
| G                             | Met             | Thr | Lys  | Arg  | G                             |
|                               | Val             | Ala | Asp  | Gly  | U                             |
|                               | Val             | Ala | Asp  | Gly  | C                             |
|                               | Val             | Ala | Glu  | Gly  | A                             |
|                               | Val             | Ala | Glu  | Gly  | G                             |

Figure 2

|              |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |     |     |
|--------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|-----|-----|
| GRVER51.SEQ  | A | T | G | A | T | G | A | A | A | C | G | C | G | A | A | A | A | G | A | A | C | G | T | G | A | T | C | T | A | C | G | G | C | C | C | A | G | A | A | C   | 40  |
| GR6.SEQ      | A | T | G | A | T | G | A | A | A | C | G | C | G | A | A | A | A | G | A | A | C | G | T | G | A | T | C | T | A | C | G | G | C | C | C | A | G | A | A | C   | 40  |
| GRVER5.SEQ   | A | T | G | A | T | G | A | A | A | C | G | C | G | A | A | A | A | G | A | A | C | G | T | G | A | T | C | T | A | C | G | G | C | C | C | A | G | A | A | C   | 40  |
| GRVER4.SEQ   | A | T | G | A | T | G | A | A | A | C | G | C | G | A | A | A | A | G | A | A | C | G | T | G | A | T | C | T | A | C | G | G | C | C | C | A | G | A | A | C   | 40  |
| GRVER3.SEQ   | A | T | G | A | T | G | A | A | A | C | G | C | G | A | A | A | A | G | A | A | C | G | T | G | A | T | C | T | A | C | G | G | C | C | C | A | G | A | A | C   | 40  |
| GRVER2.SEQ   | A | T | G | A | T | G | A | A | A | C | G | C | G | A | A | A | A | G | A | A | C | G | T | C | A | T | C | T | A | C | G | G | C | C | C | A | G | A | G | C   | 40  |
| GRVER1.SEQ   | A | T | G | A | T | G | A | A | A | C | G | C | G | A | A | A | A | G | A | A | C | G | T | C | A | T | C | T | A | C | G | G | C | C | C | A | G | A | G | C   | 40  |
| YG81-6G1.SEQ | A | T | G | A | T | G | A | A | G | C | G | A | G | A | G | A | A | A | A | A | T | G | T | T | A | T | A | T | A | T | G | G | A | C | C | C | G | A | A | C   | 40  |
| RDVER1.SEQ   | A | T | G | A | T | G | A | A | G | C | G | T | G | A | G | A | A | A | A | A | T | G | T | G | A | T | T | A | T | G | G | T | C | C | T | G | A | A | C | 40  |     |
| RDVER2.SEQ   | A | T | G | A | T | G | A | A | G | C | G | T | G | A | G | A | A | A | A | A | T | G | T | G | A | T | T | A | T | G | G | T | C | C | T | G | A | A | C | 40  |     |
| RDVER3.SEQ   | A | T | G | A | T | G | A | A | G | C | G | T | G | A | G | A | A | A | A | A | T | G | T | C | A | T | C | T | A | T | G | G | C | C | C | T | G | A | G | C   | 40  |
| RDVER4.SEQ   | A | T | G | A | T | G | A | A | G | C | G | T | G | A | G | A | A | A | A | A | T | G | T | C | A | T | C | T | A | T | G | G | C | C | C | T | G | A | G | C   | 40  |
| RDVER5.SEQ   | A | T | G | A | T | G | A | A | G | C | G | T | G | A | G | A | A | A | A | A | T | G | T | C | A | T | C | T | A | T | G | G | C | C | C | T | G | A | G | C   | 40  |
| RD7.SEQ      | A | T | G | A | T | G | A | A | G | C | G | T | G | A | G | A | A | A | A | A | T | G | T | C | A | T | C | T | A | T | G | G | C | C | C | T | G | A | G | C   | 40  |
| RDVER51.SEQ  | A | T | G | A | T | G | A | A | G | C | G | T | G | A | G | A | A | A | A | A | T | G | T | C | A | T | C | T | A | T | G | G | C | C | C | T | G | A | G | C   | 40  |
| RDVER52.SEQ  | A | T | G | A | T | G | A | A | G | C | G | T | G | A | G | A | A | A | A | A | T | G | T | C | A | T | C | T | A | T | G | G | C | C | C | T | G | A | G | C   | 40  |
| RD1561H9.SEQ | A | T | G | A | T | A | A | A | G | C | G | T | G | A | G | A | A | A | A | A | T | G | T | C | A | T | C | T | A | T | G | G | C | C | C | T | G | A | G | C   | 40  |
| GRVER51.SEQ  | C | A | C | T | G | C | A | T | C | C | A | C | T | G | G | A | A | G | A | C | C | T | C | A | C | C | G | C | T | G | G | T | G | A | G | A | T | G | C | T   | 80  |
| GR6.SEQ      | C | A | C | T | G | C | A | T | C | C | A | C | T | G | G | A | A | G | A | C | C | T | C | A | C | C | G | C | T | G | G | T | G | A | G | A | T | G | C | T   | 80  |
| GRVER5.SEQ   | C | A | C | T | G | C | A | T | C | C | A | C | T | G | G | A | A | G | A | C | C | T | C | A | C | C | G | C | T | G | G | T | G | A | G | A | T | G | C | T   | 80  |
| GRVER4.SEQ   | C | A | C | T | G | C | A | T | C | C | A | C | T | G | G | A | A | G | A | C | C | T | C | A | C | C | G | C | T | G | G | T | G | A | G | A | T | G | C | T   | 80  |
| GRVER3.SEQ   | C | A | C | T | G | C | A | T | C | C | A | C | T | G | G | A | A | G | A | C | C | T | C | A | C | C | G | C | T | G | G | T | G | A | G | A | T | G | C | T   | 80  |
| GRVER2.SEQ   | C | T | C | T | G | C | A | C | C | C | A | T | T | G | G | A | A | G | A | C | C | T | G | A | C | C | G | C | T | G | G | T | G | A | G | A | T | G | C | T   | 80  |
| GRVER1.SEQ   | C | T | C | T | G | C | A | C | C | C | A | T | T | G | G | A | A | G | A | C | C | T | G | A | C | C | G | C | C | G | G | T | G | A | G | A | T | G | C | T   | 80  |
| YG81-6G1.SEQ | C | C | T | A | C | A | C | C | C | C | T | T | G | G | A | A | G | A | C | T | T | A | A | C | A | G | C | T | G | G | A | G | A | A | A | T | G | C | T | 80  |     |
| RDVER1.SEQ   | C | A | T | T | G | C | A | T | C | C | T | C | T | G | G | A | G | G | A | T | T | T | G | A | C | T | G | C | T | G | G | C | G | A | A | A | T | G | C | T   | 80  |
| RDVER2.SEQ   | C | A | T | T | G | C | A | T | C | C | T | C | T | G | G | A | G | G | A | T | T | T | G | A | C | T | G | C | C | G | G | C | G | A | A | A | T | G | C | T   | 80  |
| RDVER3.SEQ   | C | T | T | T | G | C | A | C | C | C | T | T | T | G | G | A | G | G | A | T | T | T | G | A | C | T | G | C | C | G | G | C | G | A | A | A | T | G | C | T   | 80  |
| RDVER4.SEQ   | C | T | T | T | G | C | A | T | C | C | T | T | T | G | G | A | G | G | A | T | T | T | G | A | C | T | G | C | C | G | G | C | G | A | A | A | T | G | C | T   | 80  |
| RDVER5.SEQ   | C | T | C | T | C | C | A | T | C | C | T | T | T | G | G | A | G | G | A | T | T | T | G | A | C | T | G | C | C | G | G | C | G | A | A | A | T | G | C | T   | 80  |
| RD7.SEQ      | C | T | C | T | C | C | A | T | C | C | T | T | T | G | G | A | G | G | A | T | T | T | G | A | C | T | G | C | C | G | G | C | G | A | A | A | T | G | C | T   | 80  |
| RDVER51.SEQ  | C | T | C | T | C | C | A | T | C | C | T | T | T | G | G | A | G | G | A | T | T | T | G | A | C | T | G | C | C | G | G | C | G | A | A | A | T | G | C | T   | 80  |
| RDVER52.SEQ  | C | T | C | T | C | C | A | T | C | C | T | T | T | G | G | A | G | G | A | T | T | T | G | A | C | T | G | C | C | G | G | C | G | A | A | A | T | G | C | T   | 80  |
| RD1561H9.SEQ | C | T | C | T | C | C | A | T | C | C | T | T | T | G | G | A | G | G | A | T | T | T | G | A | C | T | G | C | C | G | G | C | G | A | A | A | T | G | C | T   | 80  |
| GRVER51.SEQ  | C | T | T | C | C | G | A | G | C | A | C | T | G | C | G | T | A | A | A | C | A | T | A | G | T | C | A | C | C | T | C | C | C | T | C | A | A | G | C | A   | 120 |
| GR6.SEQ      | C | T | T | C | C | G | A | G | C | A | C | T | G | C | G | T | A | A | A | C | A | T | A | G | T | C | A | C | C | T | C | C | C | T | C | A | A | G | C | A   | 120 |
| GRVER5.SEQ   | C | T | T | C | C | G | A | G | C | A | C | T | G | C | G | T | A | A | A | C | A | T | A | G | T | C | A | C | C | T | C | C | C | T | C | A | A | G | C | A   | 120 |
| GRVER4.SEQ   | C | T | T | C | C | G | T | G | C | A | C | T | G | C | G | T | A | A | A | C | A | T | A | G | T | C | A | C | C | T | C | C | C | T | C | A | A | G | C | T   | 120 |
| GRVER3.SEQ   | G | T | T | C | C | G | T | G | C | C | C | T | G | C | G | T | A | A | A | C | A | T | A | G | C | C | A | C | C | T | G | C | C | T | C | A | A | G | C | T   | 120 |
| GRVER2.SEQ   | G | T | T | C | C | G | T | G | C | T | C | T | G | C | G | T | A | A | A | C | A | T | T | C | T | C | A | C | T | T | G | C | C | T | C | A | A | G | C | C   | 120 |
| GRVER1.SEQ   | G | T | T | C | C | G | T | G | C | T | C | T | G | C | G | T | A | A | A | C | A | T | T | C | T | C | A | C | T | T | G | C | C | T | C | A | A | G | C | C   | 120 |
| YG81-6G1.SEQ | C | T | T | C | C | G | T | G | C | C | C | T | T | C | G | A | A | A | A | C | A | T | T | C | T | C | A | T | T | T | A | C | G | C | A | G | G | C | T | 120 |     |
| RDVER1.SEQ   | G | T | T | T | C | G | C | G | C | C | T | T | G | C | G | C | A | A | G | C | A | C | A | G | C | C | A | T | C | T | G | C | C | A | C | A | G | G | C | T   | 120 |
| RDVER2.SEQ   | G | T | T | T | C | G | C | G | C | C | T | T | G | C | G | C | A | A | G | C | A | C | A | G | C | C | A | T | C | T | G | C | C | A | C | A | A | G | C | T   | 120 |
| RDVER3.SEQ   | G | T | T | T | C | G | C | G | C | T | T | T | G | C | G | T | A | A | G | C | A | C | T | C | T | C | A | T | T | T | G | C | C | T | C | A | A | G | C | C   | 120 |
| RDVER4.SEQ   | G | T | T | T | C | G | T | G | C | T | T | T | G | C | G | T | A | A | A | C | A | C | T | C | T | C | A | T | T | T | G | C | C | T | C | A | A | G | C | C   | 120 |
| RDVER5.SEQ   | G | T | T | T | C | G | T | G | C | T | C | T | C | C | G | C | A | A | G | C | A | C | T | C | T | C | A | T | T | T | G | C | C | T | C | A | A | G | C | C   | 120 |
| RD7.SEQ      | G | T | T | T | C | G | T | G | C | T | C | T | C | C | G | C | A | A | G | C | A | C | T | C | T | T | A | T | T | T | G | C | C | T | C | A | A | G | C | C   | 120 |
| RDVER51.SEQ  | G | T | T | T | C | G | T | G | C | T | C | T | C | C | G | C | A | A | G | C | A | C | T | C | T | C | A | T | T | T | G | C | C | T | C | A | A | G | C | C   | 120 |
| RDVER52.SEQ  | G | T | T | T | C | G | T | G | C | T | C | T | C | C | G | C | A | A | G | C | A | C | T | C | T | C | A | T | T | T | G | C | C | T | C | A | A | G | C | C   | 120 |
| RD1561H9.SEQ | G | T | T | T | C | G | T | G | C | T | C | T | C | C | G | C | A | A | G | C | A | C | T | C | T | C | A | T | T | T | G | C | C | T | C | A | A | G | C | C   | 120 |

Figure 2 (c. t.)

|              |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |     |     |     |
|--------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|-----|-----|-----|
| GRVER51.SEQ  | C | T | C | G | T | G | G | A | C | G | T | C | G | T | G | G | A | G | A | C | G | A | G | A | G | C | C | T | C | T | C | C | T | A | C | A | A | A | G   | 160 |     |
| GR6.SEQ      | C | T | C | G | T | G | G | A | C | G | T | C | G | T | G | G | A | G | A | C | G | A | G | A | A | C | C | T | C | T | C | C | T | A | C | A | A | A | G   | 160 |     |
| GRVER5.SEQ   | C | T | C | G | T | G | G | A | C | G | T | C | G | T | G | G | A | G | A | C | G | A | G | A | G | C | C | T | C | T | C | C | T | A | C | A | A | A | G   | 160 |     |
| GRVER4.SEQ   | C | T | C | G | T | G | G | A | C | G | T | C | G | T | G | G | A | G | A | C | G | A | G | A | G | C | C | T | C | T | C | T | T | A | C | A | A | A | G   | 160 |     |
| GRVER3.SEQ   | C | T | C | G | T | G | G | A | C | G | T | C | G | T | G | G | G | T | G | A | C | G | A | G | A | G | C | C | T | G | T | C | T | T | A | C | A | A | A   | G   | 160 |
| GRVER2.SEQ   | C | T | G | G | T | C | G | A | T | G | T | C | G | T | G | G | C | G | A | C | G | A | G | A | G | C | T | T | G | T | C | T | T | A | T | A | A | G | 160 |     |     |
| GRVER1.SEQ   | C | T | G | G | T | G | G | A | T | G | T | C | G | T | G | G | C | G | A | C | G | A | A | A | G | C | T | T | G | T | C | T | T | A | T | A | A | G | 160 |     |     |
| YG81-6G1.SEQ | T | T | A | G | T | A | G | A | T | G | T | G | G | T | T | G | G | C | G | A | C | G | A | A | T | C | G | C | T | T | T | C | C | T | A | T | A | A | G   | 160 |     |
| RDVER1.SEQ   | T | T | G | G | T | C | G | A | C | G | T | G | G | T | C | G | G | T | G | A | T | G | A | G | T | C | T | C | T | G | A | G | C | T | A | C | A | A | A   | G   | 160 |
| RDVER2.SEQ   | T | T | G | G | T | C | G | A | C | G | T | G | G | T | C | G | G | T | G | A | T | G | A | A | T | C | T | C | T | G | A | G | C | T | A | C | A | A | A   | G   | 160 |
| RDVER3.SEQ   | T | T | G | G | T | C | G | A | T | G | T | G | G | T | C | G | G | C | G | A | T | G | A | A | T | C | T | T | T | G | A | G | C | T | A | T | A | A | G   | 160 |     |
| RDVER4.SEQ   | T | T | G | G | T | C | G | A | T | G | T | G | G | T | C | G | G | C | G | A | T | G | A | A | T | C | T | T | T | G | A | G | C | T | A | C | A | A | G   | 160 |     |
| RDVER5.SEQ   | T | T | G | G | T | C | G | A | T | G | T | G | G | T | C | G | G | C | G | A | T | G | A | A | T | C | T | T | T | G | A | G | C | T | A | C | A | A | G   | 160 |     |
| RD7.SEQ      | T | T | G | G | T | C | G | A | T | G | T | G | G | T | C | G | G | C | G | A | T | G | A | A | T | C | T | T | T | G | A | G | C | T | A | C | A | A | G   | 160 |     |
| RDVER51.SEQ  | T | T | G | G | T | C | G | A | T | G | T | G | G | T | C | G | G | C | G | A | T | G | A | A | T | C | T | T | T | G | A | G | C | T | A | C | A | A | G   | 160 |     |
| RDVER52.SEQ  | T | T | G | G | T | C | G | A | T | G | T | G | G | T | C | G | G | C | G | A | T | G | A | A | T | C | T | T | T | G | A | G | C | T | A | C | A | A | G   | 160 |     |
| RD1561H9.SEQ | T | T | G | G | T | C | G | A | T | G | T | G | G | T | C | G | G | C | G | A | T | G | A | A | T | C | T | T | T | G | A | G | C | T | A | C | A | A | G   | 160 |     |

|              |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |     |     |     |
|--------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|-----|-----|-----|
| GRVER51.SEQ  | A | A | T | T | T | T | C | G | A | A | G | C | T | A | C | T | G | T | G | C | T | G | T | T | G | G | C | C | C | A | A | A | G | C | C | T | C | C | A | 200 |     |     |
| GR6.SEQ      | A | A | T | T | T | T | T | C | G | A | A | G | C | T | A | C | T | G | T | G | C | T | G | T | T | G | G | C | C | C | A | A | A | G | C | C | T | C | C | A   | 200 |     |
| GRVER5.SEQ   | A | A | T | T | T | T | T | C | G | A | A | G | C | T | A | C | T | G | T | G | C | T | G | T | T | G | G | C | C | C | A | A | A | G | C | C | T | C | C | A   | 200 |     |
| GRVER4.SEQ   | A | A | T | T | T | T | T | C | G | A | A | G | C | T | A | C | T | G | T | G | C | T | G | T | T | G | G | C | C | C | A | A | A | G | C | C | T | C | C | A   | 200 |     |
| GRVER3.SEQ   | A | A | T | T | T | T | T | C | G | A | A | G | C | T | A | C | T | G | T | G | C | T | G | T | T | G | G | C | C | C | A | A | A | G | C | C | T | G | C | A   | 200 |     |
| GRVER2.SEQ   | A | A | T | T | T | T | T | C | G | A | A | G | C | T | A | C | T | G | T | G | C | T | G | T | T | G | G | C | C | C | A | A | A | G | C | T | C | T | G | C   | A   | 200 |
| GRVER1.SEQ   | A | G | T | T | T | T | T | C | G | A | A | G | C | T | A | C | T | G | T | C | C | T | G | T | T | G | G | C | C | C | A | G | T | C | T | C | T | G | C | A   | 200 |     |
| YG81-6G1.SEQ | A | G | T | T | T | T | T | T | G | A | A | G | C | G | A | C | A | G | T | C | C | T | C | C | T | A | G | C | G | C | A | A | A | G | T | C | T | C | C | A   | 200 |     |
| RDVER1.SEQ   | A | A | T | T | C | T | T | T | G | A | G | G | C | A | A | C | C | G | T | G | T | T | G | C | T | G | G | C | T | C | A | A | A | G | C | T | T | G | C | A   | 200 |     |
| RDVER2.SEQ   | A | G | T | T | C | T | T | T | G | A | G | G | C | A | A | C | C | G | T | G | T | T | G | C | T | G | G | C | T | C | A | G | A | G | C | T | T | G | C | A   | 200 |     |
| RDVER3.SEQ   | A | G | T | T | T | T | T | T | G | A | G | G | C | A | A | C | C | G | T | C | T | T | G | C | T | G | G | C | T | C | A | G | T | C | T | T | T | G | C | A   | 200 |     |
| RDVER4.SEQ   | A | G | T | T | T | T | T | T | G | A | G | G | C | A | A | C | C | G | T | C | T | T | G | C | T | G | G | C | T | C | A | G | T | C | C | T | T | T | G | C   | A   | 200 |
| RDVER5.SEQ   | A | G | T | T | T | T | T | T | G | A | G | G | C | A | A | C | C | G | T | C | T | T | G | C | T | G | G | C | T | C | A | G | T | C | C | C | T | C | C | A   | 200 |     |
| RD7.SEQ      | A | G | T | T | T | T | T | T | G | A | G | G | C | A | A | C | C | G | T | C | T | T | G | C | T | G | G | C | T | C | A | G | T | C | C | C | T | C | C | A   | 200 |     |
| RDVER51.SEQ  | A | G | T | T | T | T | T | T | G | A | G | G | C | A | A | C | C | G | T | C | T | T | G | C | T | G | G | C | T | C | A | G | T | C | C | C | T | C | C | A   | 200 |     |
| RDVER52.SEQ  | A | G | T | T | T | T | T | T | G | A | G | G | C | A | A | C | C | G | T | C | T | T | G | C | T | G | G | C | T | C | A | G | T | C | C | C | T | C | C | A   | 200 |     |
| RD1561H9.SEQ | A | G | T | T | T | T | T | T | G | A | G | G | C | A | A | C | C | G | T | C | T | T | G | C | T | G | G | C | T | C | A | G | T | C | C | C | T | C | C | A   | 200 |     |

|              |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |     |     |
|--------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|-----|-----|
| GRVER51.SEQ  | T | A | A | T | T | G | T | G | G | G | T | A | C | A | A | A | A | T | G | A | A | C | G | A | T | G | T | G | G | T | G | A | G | C | A | T | T | T | G | T | 240 |     |
| GR6.SEQ      | T | A | A | T | T | G | T | G | G | G | T | A | C | A | A | A | A | A | T | G | A | A | C | G | A | T | G | T | G | G | T | G | A | G | C | A | T | T | T | G | T   | 240 |
| GRVER5.SEQ   | T | A | A | T | T | G | T | G | G | G | T | A | C | A | A | A | A | A | T | G | A | A | C | G | A | T | G | T | G | G | T | G | A | G | C | A | T | T | T | G | T   | 240 |
| GRVER4.SEQ   | T | A | A | T | T | G | T | G | G | A | T | A | C | A | A | A | A | A | T | G | A | A | C | G | A | T | G | T | G | G | T | G | A | G | C | A | T | T | T | G | T   | 240 |
| GRVER3.SEQ   | T | A | A | T | T | G | T | G | G | T | T | A | C | A | A | A | A | A | T | G | A | A | C | G | A | T | G | T | G | G | T | G | A | G | C | A | T | C | T | G | T   | 240 |
| GRVER2.SEQ   | T | A | A | T | T | G | C | G | G | T | T | A | C | A | A | A | A | A | T | G | A | A | C | G | A | T | G | T | G | G | T | C | A | G | C | A | T | T | T | G | T   | 240 |
| GRVER1.SEQ   | T | A | A | T | T | G | C | G | G | T | T | A | C | A | A | A | A | A | T | G | A | A | C | G | A | T | G | T | G | G | T | C | A | G | C | A | T | T | T | G | T   | 240 |
| YG81-6G1.SEQ | C | A | A | T | T | G | T | G | G | A | T | A | C | A | A | G | A | T | G | A | A | T | G | A | T | G | T | A | G | T | G | T | C | G | A | T | C | T | G | C | 240 |     |
| RDVER1.SEQ   | C | A | A | C | T | G | T | G | G | C | T | A | T | A | A | G | A | T | G | A | A | T | G | A | C | G | T | C | G | T | G | T | C | T | A | T | C | T | G | C | 240 |     |
| RDVER2.SEQ   | C | A | A | C | T | G | T | G | G | C | T | A | T | A | A | G | A | T | G | A | A | T | G | A | C | G | T | C | G | T | G | T | C | T | A | T | C | T | G | C | 240 |     |
| RDVER3.SEQ   | T | A | A | T | T | G | C | G | G | C | T | A | C | A | A | G | A | T | G | A | A | C | G | A | C | G | T | C | G | T | C | T | C | T | A | T | T | T | G | T | 240 |     |
| RDVER4.SEQ   | T | A | A | T | T | G | T | G | G | C | T | A | C | A | A | G | A | T | G | A | A | C | G | A | C | G | T | C | G | T | C | T | C | C | A | T | T | T | G | T | 240 |     |
| RDVER5.SEQ   | C | A | A | T | T | G | T | G | G | C | T | A | C | A | A | G | A | T | G | A | A | C | G | A | C | G | T | C | G | T | T | A | G | T | A | T | C | T | G | T | 240 |     |
| RD7.SEQ      | C | A | A | T | T | G | T | G | G | C | T | A | C | A | A | G | A | T | G | A | A | C | G | A | C | G | T | C | G | T | T | A | G | T | A | T | C | T | G | T | 240 |     |
| RDVER51.SEQ  | C | A | A | T | T | G | T | G | G | C | T | A | C | A | A | G | A | T | G | A | A | C | G | A | C | G | T | C | G | T | T | A | G | T | A | T | C | T | G | T | 240 |     |
| RDVER52.SEQ  | C | A | A | T | T | G | T | G | G | C | T | A | C | A | A | G | A | T | G | A | A | C | G | A | C | G | T | C | G | T | T | A | G | T | A | T | C | T | G | T | 240 |     |
| RD1561H9.SEQ | C | A | A | T | T | G | T | G | G | C | T | A | C | A | A | G | A | T | G | A | A | C | G | A | C | G | T | C | G | T | T | A | G | T | A | T | C | T | G | T | 240 |     |





Figure 2 (cont.)

|              |   |     |
|--------------|---|-----|
| GRVER51.SEQ  | A A A C C T C A A A T C G T C T T T A C T A C C A A A A A A C A T C T T G A A T A | 400 |
| GR6.SEQ      | A A A C C T C A A A T C G T C T T T A C T A C C A A A A A A C A T C T T G A A T A | 400 |
| GRVER5.SEQ   | A A A C C T C A A A T C G T C T T T A C T A C C A A A A A A C A T C T T G A A T A | 400 |
| GRVER4.SEQ   | A A A C C T C A A A T C G T C T T T A C T A C C A A A A A A T A T C C T G A A T A | 400 |
| GRVER3.SEQ   | A A A C C T C A A A T C G T C T T T A C T A C C A A A A A A C A T C C T G A A T A | 400 |
| GRVER2.SEQ   | A A A C C T C A A A T C G T G T T T A C T A C C A A G A A C A T T C T G A A T A   | 400 |
| GRVER1.SEQ   | A A A C C T C A A A T C G T G T T T A C T A C C A A G A A C A T T C T G A A T A   | 400 |
| YG81-6G1.SEQ | A A A C C A C A A A T A G T T T T A C G A C A A A G A A C A T T T T A A A T A     | 400 |
| RDVER1.SEQ   | A A G C C A C A G A T T G T C T T C A C C A C T A A A A A T A T C T T G A A C A   | 400 |
| RDVER2.SEQ   | A A G C C A C A G A T T G T C T T C A C C A C T A A A A A T A T C T T G A A C A   | 400 |
| RDVER3.SEQ   | A A G C C A C A G A T T G T C T T C A C C A C T A A G A A T A T T T T G A A C A   | 400 |
| RDVER4.SEQ   | A A G C C A C A G A T T G T C T T C A C C A C T A A G A A T A T T C T G A A C A   | 400 |
| RDVER5.SEQ   | A A G C C A C A G A T T G T C T T C A C C A C T A A G A A T A T T C T G A A C A   | 400 |
| RD7.SEQ      | A A G C C A C A G A T T G T C T T C A C C A C T A A G A A T A T T C T G A A C A   | 400 |
| RDVER51.SEQ  | A A G C C A C A G A T T G T C T T C A C C A C T A A G A A T A T T C T G A A C A   | 400 |
| RDVER52.SEQ  | A A G C C A C A G A T T G T C T T C A C C A C T A A G A A T A T T C T G A A C A   | 400 |
| RD1561H9.SEQ | A A G C C A C A G A T T G T C T T C A C C A C T A A G A A T A T T C T G A A C A   | 400 |
|              |   |     |
| GRVER51.SEQ  | A G G T C T T G G A A G T C C A G T C T C G T A C T A A C T T C A T C A A A C G   | 440 |
| GR6.SEQ      | A G G T C T T G G A A G T C C A G T C T C G T A C T A A C T T C A T C A A A C G   | 440 |
| GRVER5.SEQ   | A G G T C T T G G A A G T C C A G T C T C G T A C T A A C T T C A T C A A A C G   | 440 |
| GRVER4.SEQ   | A G G T C T T G G A A G T C C A G T C T C G T A C T A A C T T C A T C A A A C G   | 440 |
| GRVER3.SEQ   | A G G T C T T G G A A G T C C A G T C T C G T A C T A A T T T C A T C A A A C G   | 440 |
| GRVER2.SEQ   | A G G T C T T G G A A G T G C A G T C T C G T A C T A A C T T C A T C A A G C G   | 440 |
| GRVER1.SEQ   | A A G T C T T G G A A G T G C A G T C T C G T A C T A A C T T C A T C A A G C G   | 440 |
| YG81-6G1.SEQ | A G G T A T T G G A G G T A C A G A G C A G A A C T A A T T T C A T A A A A A G   | 440 |
| RDVER1.SEQ   | A G G T G C T G G A G G T C C A A A G C C G C A C C A A T T T T A T T A A A C G   | 440 |
| RDVER2.SEQ   | A A G T G C T G G A G G T C C A A A G C C G C A C C A A T T T T A T T A A A C G   | 440 |
| RDVER3.SEQ   | A A G T G C T G G A A G T C C A A A G C C G C A C C A A C T T T A T T A A G C G   | 440 |
| RDVER4.SEQ   | A A G T C C T G G A A G T C C A A A G C C G C A C C A A C T T T A T T A A G C G   | 440 |
| RDVER5.SEQ   | A A G T C C T G G A A G T C C A A A G C C G C A C C A A C T T T A T T A A G C G   | 440 |
| RD7.SEQ      | A A G T C C T G G A A G T C C A A A G C C G C A C C A A C T T T A T T A A G C G   | 440 |
| RDVER51.SEQ  | A A G T C C T G G A A G T C C A A A G C C G C A C C A A C T T T A T T A A G C G   | 440 |
| RDVER52.SEQ  | A A G T C C T G G A A G T C C A A A G C C G C A C C A A C T T T A T T A A G C G   | 440 |
| RD1561H9.SEQ | A A G T C C T G G A A G T C C A A A G C C G C A C C A A C T T T A T T A A G C G   | 440 |
|              |   |     |
| GRVER51.SEQ  | C A T C A T T A T T C T G G A T A C C G T C G A A A A C A T C C A C G G C T G T   | 480 |
| GR6.SEQ      | C A T C A T T A T T C T G G A T A C C G T C G A A A A C A T C C A C G G C T G T   | 480 |
| GRVER5.SEQ   | C A T C A T T A T T C T G G A T A C C G T C G A A A A C A T C C A C G G C T G T   | 480 |
| GRVER4.SEQ   | C A T C A T T A T T C T G G A T A C C G T C G A A A A C A T C C A T G G C T G T   | 480 |
| GRVER3.SEQ   | C A T T A T T A T T C T G G A T A C C G T C G A A A A C A T C C A C G G C T G T   | 480 |
| GRVER2.SEQ   | C A T T A T C A T T C T G G A T A C C G T C G A G A A T A T C C A C G G C T G T   | 480 |
| GRVER1.SEQ   | C A T T A T C A T T C T G G A T A C C G T C G A G A A T A T C C A C G G C T G T   | 480 |
| YG81-6G1.SEQ | G A T C A T C A T A C T T G A T A C T G T A G A A A A C A T A C A C G G T T G T   | 480 |
| RDVER1.SEQ   | T A T C A T T A T C T T G G A C A C T G T G G A A A A C A T T C A T G G T T G C   | 480 |
| RDVER2.SEQ   | T A T C A T T A T C T T G G A C A C T G T G G A A A A C A T T C A T G G T T G C   | 480 |
| RDVER3.SEQ   | T A T C A T C A T C T T G G A C A C T G T G G A G A A T A T T C A T G G T T G C   | 480 |
| RDVER4.SEQ   | T A T C A T C A T C T T G G A C A C T G T G G A G A A T A T T C A C G G T T G C   | 480 |
| RDVER5.SEQ   | T A T C A T C A T C T T G G A C A C T G T G G A G A A T A T T C A C G G T T G C   | 480 |
| RD7.SEQ      | T A T C A T C A T C T T G G A C A C T G T G G A G A A T A T T C A C G G T T G C   | 480 |
| RDVER51.SEQ  | T A T C A T C A T C T T G G A C A C T G T G G A G A A T A T T C A C G G T T G C   | 480 |
| RDVER52.SEQ  | T A T C A T C A T C T T G G A C A C T G T G G A G A A T A T T C A C G G T T G C   | 480 |
| RD1561H9.SEQ | T A T C A T C A T C T T G G A C A C T G T G G A G A A T A T T C A C G G T T G C   | 480 |

Figure 2 (cont.)

GRVER51.SEQ G A G A G C C T C C C T A A C T T C A T C T C T C G T T A C A G C G A T G G T A 520  
GR6.SEQ G A G A G C C T C C C T A A C T T C A T C T C T C G T T A C A G C G A T G G T A 520  
GRVER5.SEQ G A G A G C C T C C C T A A C T T C A T C T C T C G T T A C A G C G A T G G T A 520  
GRVER4.SEQ G A G A G C C T G C C T A A C T T C A T C T C T C G T T A C A G C G A T G G T A 520  
GRVER3.SEQ G A G A G C T T G C C T A A C T T T A T C T C T C G T T A C A G C G A T G G T A 520  
GRVER2.SEQ G A G A G C T T G C C A A A C T T T A T T T C T C G T T A T A G C G A C G G T A 520  
GRVER1.SEQ G A A A G C T T G C C A A A C T T T A T T T C T C G T T A T A G C G A C G G T A 520  
YG81-6G1.SEQ G A A A G T C T T C C C A A T T T T A T T T C T C G T T A T T C G G A T G G A A 520  
RDVER1.SEQ G A G T C T C T G C C T A A T T T C A T C A G C C G C T A C T C T G A T G G C A 520  
RDVER2.SEQ G A A T C T C T G C C T A A T T T C A T C A G C C G C T A C T C T G A T G G C A 520  
RDVER3.SEQ G A A T C T C T G C C T A A T T T C A T T A G C C G C T A T T C T G A C G G C A 520  
RDVER4.SEQ G A A T C T T T G C C T A A T T T T A T T A G C C G C T A T T C A G A C G G A A 520  
RDVER5.SEQ G A A T C T T T G C C T A A T T T C A T C T C T C G C T A T T C A G A C G G C A 520  
RD7.SEQ G A A T C T T T G C C T A A T T T C A T C T C T C G C T A T T C A G A C G G C A 520  
RDVER51.SEQ G A A T C T T T G C C T A A T T T C A T C T C T C G C T A T T C A G A C G G C A 520  
RDVER52.SEQ G A A T C T T T G C C T A A T T T C A T C T C T C G C T A T T C A G A C G G C A 520  
RD1561H9.SEQ G A A T C T T T G C C T A A T T T C A T C T C T C G C T A T T C A G A C G G C A 520

GRVER51.SEQ A T A T C G C T A A T T T C A A G C C C T T G C A T T T T G A T C C A G T C G A 560  
GR6.SEQ A T A T C G C T A A T T T C A A G C C C T T G C A T T T T G A T C C A G T C G A 560  
GRVER5.SEQ A T A T C G C T A A T T T C A A G C C C T T G C A T T T T G A T C C A G T C G A 560  
GRVER4.SEQ A T A T C G C T A A T T T C A A A C C A C T G C A T T T T G A T C C A G T C G A 560  
GRVER3.SEQ A T A T C G C T A A T T T C A A G C C A C T G C A T T T T G A T C C A G T C G A 560  
GRVER2.SEQ A T A T C G C T A A C T T C A A G C C C T G C A T T T T G A T C C A G T G G A 560  
GRVER1.SEQ A T A T C G C T A A C T T C A A G C C T C T G C A T T T T G A T C C A G T G G A 560  
YG81-6G1.SEQ A T A T T G C C A A C T T C A A A C C T T T A C A T T T C G A T C C T G T T G A 560  
RDVER1.SEQ A C A T T G C C A A T T T T A A A C C A T T G C A C T T C G A C C C T G T C G A 560  
RDVER2.SEQ A C A T T G C C A A T T T T A A A C C A T T G C A C T T C G A C C C T G T C G A 560  
RDVER3.SEQ A C A T C G C C A A C T T T A A A C C T T T G C A T T T C G A C C C T G T G G A 560  
RDVER4.SEQ A C A T C G C C A A C T T T A A G C C T C T C A T T T C G A C C C T G T G G A 560  
RDVER5.SEQ A C A T C G C A A A C T T T A A A C C A C T C C A C T T C G A C C C T G T G G A 560  
RD7.SEQ A C A T C G C A A A C T T T A A A C C A C T C C A C T T C G A C C C T G T G G A 560  
RDVER51.SEQ A C A T C G C A A A C T T T A A A C C A C T C C A C T T C G A C C C T G T G G A 560  
RDVER52.SEQ A C A T C G C A A A C T T T A A A C C A C T C C A C T T C G A C C C T G T G G A 560  
RD1561H9.SEQ A C A T C G C A A A C T T T A A A C C A C T C C A C T T C G A C C C T G T G G A 560

GRVER51.SEQ G C A A G T G G C C G C T A T T T T G T G C T C C T C C G G C A C C A C T G G T 600  
GR6.SEQ G C A A G T G G C C G C T A T T T T G T G C T C C T C C G G C A C C A C T G G T 600  
GRVER5.SEQ G C A A G T G G C C G C T A T T T T G T G C T C C T C C G G C A C C A C T G G T 600  
GRVER4.SEQ G C A A G T G G C C G C T A T T T T G T G C T C T T C C G G C A C C A C T G G T 600  
GRVER3.SEQ G C A G G T C G C C G C T A T T T T G T G C T C T C T G G C A C C A C T G G T 600  
GRVER2.SEQ G C A A G T C G C C G C T A T T T T G T G C T C T A G C G G C A C C A C G G T 600  
GRVER1.SEQ G C A A G T C G C C G C T A T T T T G T G C T C T A G C G G C A C T A C C G G T 600  
YG81-6G1.SEQ G C A A G T G G C A G C T A T C T T A T G T T C G T C A G G C A C T A C T G G A 600  
RDVER1.SEQ A C A G G T G G C T G C C A T C C T G T G T A G C T C T G G T A C C A C T G G C 600  
RDVER2.SEQ A C A G G T G G C T G C C A T C C T G T G T A G C T C T G G T A C T A C T G G C 600  
RDVER3.SEQ A C A A G T G G C T G C T A T C C T G T G T A G C A G C G G T A C T A C T G G C 600  
RDVER4.SEQ A C A A G T T G C T G C A A T C C T G T G T A G C A G C G G T A C T A C T G G A 600  
RDVER5.SEQ A C A A G T T G C A G C C A T T C T G T G T A G C A G C G G T A C T A C T G G A 600  
RD7.SEQ A C A A G T T G C A G C C A T T C T G T G T A G C A G C G G T A C T A C T G G A 600  
RDVER51.SEQ A C A A G T T G C A G C C A T T C T G T G T A G C A G C G G T A C T A C T G G A 600  
RDVER52.SEQ A C A A G T T G C A G C C A T T C T G T G T A G C A G C G G T A C T A C T G G A 600  
RD1561H9.SEQ A C A A G T T G C A G C C A T T C T G T G T A G C A G C G G T A C T A C T G G A 600

Figure 2 (cont.)

GRVER51.SEQ T T G C C T A A A G G T G T C A T G C A G A C T C A C C A G A A T A T C T G T G 640  
GR6.SEQ T T G C C T A A A G G T G T C A T G C A G A C T C A C C A G A A T A T C T G T G 640  
GRVER5.SEQ T T G C C T A A A G G T G T C A T G C A G A C T C A C C A G A A T A T C T G T G 640  
GRVER4.SEQ T T G C C T A A A G G T G T C A T G C A G A C T C A C C A G A A T A T C T G T G 640  
GRVER3.SEQ T T G C C T A A A G G T G T C A T G C A G A C T C A C C A G A A T A T C T G T G 640  
GRVER2.SEQ C T G C C T A A A G G C G T G A T G C A G A C T C A C C A A A A T A T C T G T G 640  
GRVER1.SEQ C T G C C T A A A G G C G T G A T G C A G A C T C A C C A A A A T A T C T G T G 640  
YG81-6G1.SEQ T T A C C G A A A G G T G T A A T G C A A A C T C A C C A A A A T A T T T G T G 640  
RDVER1.SEQ T T G C C A A A G G G T G T C A T G C A A A C C C A T C A G A A C A T T T G T G 640  
RDVER2.SEQ T T G C C A A A G G G T G T C A T G C A A A C C C A T C A G A A C A T T T G C G 640  
RDVER3.SEQ C T C C C A A A G G G C G T C A T G C A G A C C C A T C A A A A C A T T T G C G 640  
RDVER4.SEQ C T C C C A A A G G G A G T C A T G C A G A C C C A T C A A A A C A T T T G C G 640  
RDVER5.SEQ C T C C C A A A G G G A G T C A T G C A G A C C C A T C A A A A C A T T T G C G 640  
RD7.SEQ C T C C C A A A G G G A G T C A T G C A G A C C C A T C A A A A C A T T T G C G 640  
RDVER51.SEQ C T C C C A A A G G G A G T C A T G C A G A C C C A T C A A A A C A T T T G C G 640  
RDVER52.SEQ C T C C C A A A G G G A G T C A T G C A G A C C C A T C A A A A C A T T T G C G 640  
RD1561H9.SEQ C T C C C A A A G G G A G T C A T G C A G A C C C A T C A A A A C A T T T G C G 640

GRVER51.SEQ T G C G T T T G A T C C A C G C T C T C G A C C C T C G T G T G G G T A C T C A 680  
GR6.SEQ T G C G T T T G A T C C A C G C T C T C G A C C C T C G T G T G G G T A C T C A 680  
GRVER5.SEQ T G C G T T T G A T C C A C G C T C T C G A C C C T C G T G T G G G T A C T C A 680  
GRVER4.SEQ T G C G T T T G A T C C A C G C T C T C G A C C C T C G T G T G G G T A C T C A 680  
GRVER3.SEQ T G C G C T T G A T C C A C G C C C T C G A C C C T C G T G T G G G T A C T C A 680  
GRVER2.SEQ T C C G C T T G A T T C A T G C C C T G G A C C C A C G T G T G G G T A C T C A 680  
GRVER1.SEQ T C C G C T T G A T T C A T G C C C T G G A C C C A C G T G T G G G T A C C C A 680  
YG81-6G1.SEQ T C C G A C T T A T A C A T G C T T T A G A C C C C A G G G C A G G A A C G C A 680  
RDVER1.SEQ T G C G T C T G A T C C A C G C T C T C G A T C C T C G C T A C G G C A C T C A 680  
RDVER2.SEQ T G C G T C T G A T C C A C G C T C T C G A T C C T C G C T A C G G C A C C C A 680  
RDVER3.SEQ T G C G T C T G A T C C A T G C T C T C G A T C C A C G C T A C G G C A C T C A 680  
RDVER4.SEQ T G C G T C T G A T C C A T G C T C T C G A T C C A C G C T A C G G C A C T C A 680  
RDVER5.SEQ T G C G T C T G A T C C A T G C T C T C G A T C C A C G C T A C G G C A C T C A 680  
RD7.SEQ T G C G T C T G A T C C A T G C T C T C G A T C C A C G C T A C G G C A C T C A 680  
RDVER51.SEQ T G C G T C T G A T C C A T G C T C T C G A T C C A C G C T A C G G C A C T C A 680  
RDVER52.SEQ T G C G T C T G A T C C A T G C T C T C G A T C C A C G C T A C G G C A C T C A 680  
RD1561H9.SEQ T G C G T C T G A T C C A T G C T C T C G A T C C A C G C T A C G G C A C T C A 680

GRVER51.SEQ A T T G A T C C C T G G C G T G A C T G T G C T G G T G T A T C T G C C T T T C 720  
GR6.SEQ A T T G A T C C C T G G C G T G A C T G T G C T G G T G T A T C T G C C T T T C 720  
GRVER5.SEQ A T T G A T C C C T G G C G T G A C T G T G C T G G T G T A T C T G C C T T T C 720  
GRVER4.SEQ A T T G A T C C C T G G C G T G A C T G T G C T G G T G T A T C T G C C T T T C 720  
GRVER3.SEQ A T T G A T C C C T G G C G T G A C T G T G C T G G T G T A T T T G C C T T T C 720  
GRVER2.SEQ G T T G A T C C C T G G C G T G A C T G T C C T G G T G T A C T T G C C A T T C 720  
GRVER1.SEQ G T T G A T C C C T G G C G T G A C T G T C C T G G T G T A C T T G C C A T T C 720  
YG81-6G1.SEQ A C T T A T T C C T G G T G T G A C A G T C T T A G T A T A T C T G C C T T T T 720  
RDVER1.SEQ A C T G A T T C C A G G T G T C A C C G T G T T G G T C T A T C T G C C T T T T 720  
RDVER2.SEQ A C T G A T T C C T G G T G T C A C C G T G T T G G T C T A T C T G C C T T T T 720  
RDVER3.SEQ G C T G A T T C C T G G T G T C A C C G T C T T G G T C T A C C T G C C T T T C 720  
RDVER4.SEQ G C T G A T T C C T G G T G T C A C C G T C T T G G T C T A C T T G C C T T T C 720  
RDVER5.SEQ G C T G A T T C C T G G T G T C A C C G T C T T G G T C T A C T T G C C T T T C 720  
RD7.SEQ G C T G A T T C C T G G T G T C A C C G T C T T G G T C T A C T T G C C T T T C 720  
RDVER51.SEQ G C T G A T T C C T G G T G T C A C C G T C T T G G T C T A C T T G C C T T T C 720  
RDVER52.SEQ G C T G A T T C C T G G T G T C A C C G T C T T G G T C T A C T T G C C T T T C 720  
RD1561H9.SEQ G C T G A T T C C T G G T G T C A C C G T C T T G G T C T A C T T G C C T T T C 720

Figure 2 (cont.)

|              |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |     |     |
|--------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|-----|-----|
| GRVER51.SEQ  | T | T | T | C | A | C | G | C | C | T | T | T | G | G | T | T | T | C | T | C | T | A | T | T | A | C | C | C | T | G | G | G | C | T | A | T | T | T | C | A   | 760 |
| GR6.SEQ      | T | T | T | C | A | C | G | C | C | T | T | T | G | G | T | T | T | C | T | C | T | A | T | T | A | C | C | C | T | G | G | G | C | T | A | T | T | T | C | A   | 760 |
| GRVER5.SEQ   | T | T | T | C | A | C | G | C | C | T | T | T | G | G | T | T | T | C | T | C | T | A | T | T | A | C | C | C | T | G | G | G | C | T | A | T | T | T | C | A   | 760 |
| GRVER4.SEQ   | T | T | T | C | A | C | G | C | C | T | T | T | G | G | T | T | T | T | C | T | A | T | T | A | C | C | C | T | G | G | G | C | T | A | T | T | T | C | A | 760 |     |
| GRVER3.SEQ   | T | T | T | C | A | C | G | C | C | T | T | T | G | G | T | T | T | T | C | T | A | T | T | A | C | C | C | T | G | G | G | C | T | A | T | T | T | C | A | 760 |     |
| GRVER2.SEQ   | T | T | T | C | A | C | G | C | C | T | T | C | G | G | T | T | T | T | C | T | A | T | T | A | C | C | C | T | G | G | G | C | T | A | T | T | T | C | A | 760 |     |
| GRVER1.SEQ   | T | T | T | C | A | C | G | C | C | T | T | C | G | G | T | T | T | T | C | T | A | T | T | A | C | C | C | T | G | G | G | C | T | A | T | T | T | C | A | 760 |     |
| YG81-6G1.SEQ | T | T | C | C | A | T | G | C | T | T | T | T | G | G | G | T | T | C | T | C | T | A | T | A | A | C | C | T | T | G | G | G | A | T | A | C | T | T | C | A   | 760 |
| RDVER1.SEQ   | T | T | C | C | A | T | G | C | T | T | T | T | G | G | C | T | T | C | C | A | C | A | T | C | A | C | T | T | T | G | G | G | T | T | A | C | T | T | T | A   | 760 |
| RDVER2.SEQ   | T | T | C | C | A | T | G | C | T | T | T | T | G | G | C | T | T | C | C | A | C | A | T | C | A | C | T | T | T | G | G | G | T | T | A | C | T | T | T | A   | 760 |
| RDVER3.SEQ   | T | T | C | C | A | T | G | C | T | T | T | C | G | G | C | T | T | C | C | A | C | A | T | T | A | C | T | T | T | G | G | G | T | T | A | C | T | T | T | A   | 760 |
| RDVER4.SEQ   | T | T | C | C | A | T | G | C | T | T | T | C | G | G | C | T | T | C | C | A | T | A | T | T | A | C | T | T | T | G | G | G | T | T | A | C | T | T | T | A   | 760 |
| RDVER5.SEQ   | T | T | C | C | A | T | G | C | T | T | T | C | G | G | C | T | T | T | C | A | T | A | T | T | A | C | T | T | T | G | G | G | T | T | A | C | T | T | T | A   | 760 |
| RD7.SEQ      | T | T | C | C | A | T | G | C | T | T | T | C | G | G | C | T | T | T | C | A | T | A | T | T | A | C | T | T | T | G | G | G | T | T | A | C | T | T | T | A   | 760 |
| RDVER51.SEQ  | T | T | C | C | A | T | G | C | T | T | T | C | G | G | C | T | T | T | C | A | T | A | T | T | A | C | T | T | T | G | G | G | T | T | A | C | T | T | T | A   | 760 |
| RDVER52.SEQ  | T | T | C | C | A | T | G | C | T | T | T | C | G | G | C | T | T | T | C | A | T | A | T | T | A | C | T | T | T | G | G | G | T | T | A | C | T | T | T | A   | 760 |
| RD1561H9.SEQ | T | T | C | C | A | T | G | C | T | T | T | C | G | G | C | T | T | T | C | A | T | A | T | T | A | C | T | T | T | G | G | G | T | T | A | C | T | T | T | A   | 760 |

|              |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |     |     |
|--------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|-----|-----|
| GRVER51.SEQ  | T | G | G | T | C | G | G | C | T | T | G | C | G | T | G | T | C | A | T | C | A | T | G | T | T | T | C | G | T | C | G | C | T | T | C | G | A | C | C | A | 800 |     |
| GR6.SEQ      | T | G | G | T | C | G | G | C | T | T | G | C | G | T | G | T | C | A | T | C | A | T | G | T | T | T | C | G | T | C | G | C | T | T | C | G | A | C | C | A | 800 |     |
| GRVER5.SEQ   | T | G | G | T | C | G | G | C | T | T | G | C | G | T | G | T | C | A | T | C | A | T | G | T | T | T | C | G | T | C | G | C | T | T | C | G | A | C | C | A | 800 |     |
| GRVER4.SEQ   | T | G | G | T | C | G | G | C | T | T | G | C | G | T | G | T | C | A | T | C | A | T | G | T | T | T | C | G | T | C | G | C | T | T | C | G | A | C | C | A | 800 |     |
| GRVER3.SEQ   | T | G | G | T | C | G | G | C | T | T | G | C | G | T | G | T | G | A | T | C | A | T | G | T | T | T | T | C | G | T | C | G | C | T | T | C | G | A | T | C | A   | 800 |
| GRVER2.SEQ   | T | G | G | T | C | G | G | T | T | T | G | C | G | C | G | T | G | A | T | C | A | T | G | T | T | T | T | C | G | T | C | G | C | T | T | C | G | A | T | C | A   | 800 |
| GRVER1.SEQ   | T | G | G | T | C | G | G | T | T | T | G | C | G | C | G | T | G | A | T | C | A | T | G | T | T | T | T | C | G | T | C | G | C | T | T | C | G | A | T | C | A   | 800 |
| YG81-6G1.SEQ | T | G | G | T | G | G | G | T | C | T | T | C | G | T | G | T | T | A | T | C | A | T | G | T | T | T | C | A | G | A | C | G | A | T | T | T | G | A | T | C | A   | 800 |
| RDVER1.SEQ   | T | G | G | T | G | G | G | C | C | T | G | C | G | T | G | T | C | A | T | T | A | T | G | T | T | T | C | G | C | C | G | T | T | T | T | G | A | T | C | C | A   | 800 |
| RDVER2.SEQ   | T | G | G | T | G | G | G | C | C | T | G | C | G | T | G | T | C | A | T | T | A | T | G | T | T | T | C | G | C | C | G | T | T | T | T | G | A | T | C | C | A   | 800 |
| RDVER3.SEQ   | T | G | G | T | C | G | G | T | C | T | G | C | G | T | G | T | C | A | T | T | A | T | G | T | T | T | C | G | C | C | G | T | T | T | T | G | A | T | C | A | 800 |     |
| RDVER4.SEQ   | T | G | G | T | C | G | G | T | C | T | G | C | G | T | G | T | C | A | T | A | T | G | T | T | T | T | C | G | C | C | G | T | T | T | T | G | A | T | C | A | 800 |     |
| RDVER5.SEQ   | T | G | G | T | C | G | G | T | C | T | G | C | G | T | C | T | G | A | T | T | A | T | G | T | T | T | T | C | G | C | C | G | T | T | T | T | G | A | T | C | A   | 800 |
| RD7.SEQ      | T | G | G | T | C | G | G | T | C | T | C | C | G | C | G | T | G | A | T | T | A | T | G | T | T | T | T | C | G | C | C | G | T | T | T | T | G | A | T | C | A   | 800 |
| RDVER51.SEQ  | T | G | G | T | C | G | G | T | C | T | C | C | G | C | G | T | G | A | T | T | A | T | G | T | T | T | T | C | G | C | C | G | T | T | T | T | G | A | T | C | A   | 800 |
| RDVER52.SEQ  | T | G | G | T | C | G | G | T | C | T | C | C | G | C | G | T | G | A | T | T | A | T | G | T | T | T | T | C | G | C | C | G | T | T | T | T | G | A | T | C | A   | 800 |
| RD1561H9.SEQ | T | G | G | T | C | G | G | T | C | T | C | C | G | C | G | T | G | A | T | T | A | T | G | T | T | T | T | C | G | C | C | G | T | T | T | T | G | A | T | C | A   | 800 |

|              |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |     |
|--------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|-----|
| GRVER51.SEQ  | A | G | A | A | G | C | C | T | T | C | T | T | G | A | A | G | G | C | T | A | T | T | C | A | A | G | A | C | T | A | C | G | A | G | G | T | G | C | G | T | 840 |
| GR6.SEQ      | A | G | A | A | G | C | C | T | T | C | T | T | G | A | A | G | G | C | T | A | T | T | C | A | A | G | A | C | T | A | C | G | A | G | G | T | G | C | G | T | 840 |
| GRVER5.SEQ   | A | G | A | A | G | C | C | T | T | C | T | T | G | A | A | G | G | C | T | A | T | T | C | A | A | G | A | C | T | A | C | G | A | G | G | T | G | C | G | T | 840 |
| GRVER4.SEQ   | A | G | A | A | G | C | C | T | T | C | T | T | G | A | A | G | G | C | T | A | T | T | C | A | A | G | A | C | T | A | C | G | A | G | G | T | G | C | G | T | 840 |
| GRVER3.SEQ   | A | G | A | A | G | C | C | T | T | C | T | T | G | A | A | G | G | C | T | A | T | T | C | A | A | G | A | C | T | A | C | G | A | G | G | T | G | C | G | T | 840 |
| GRVER2.SEQ   | A | G | A | A | G | C | C | T | T | T | C | T | G | A | A | G | G | C | C | A | T | T | C | A | A | G | A | C | T | A | C | G | A | G | G | T | C | C | G | T | 840 |
| GRVER1.SEQ   | A | G | A | A | G | C | T | T | T | T | C | T | G | A | A | G | G | C | C | A | T | T | C | A | A | G | A | C | T | A | C | G | A | G | G | T | C | C | G | T | 840 |
| YG81-6G1.SEQ | A | G | A | A | G | C | A | T | T | T | C | T | A | A | A | A | G | C | T | A | T | T | C | A | G | G | A | T | T | A | T | G | A | A | G | T | T | C | G | A | 840 |
| RDVER1.SEQ   | G | G | A | G | G | C | C | T | T | C | T | T | G | A | A | A | G | C | T | A | T | C | C | A | A | G | A | T | T | A | T | G | A | A | G | T | G | C | G | C | 840 |
| RDVER2.SEQ   | G | G | A | G | G | C | T | T | T | C | T | T | G | A | A | A | G | C | T | A | T | C | C | A | A | G | A | T | T | A | T | G | A | A | G | T | G | C | G | C | 840 |
| RDVER3.SEQ   | G | G | A | G | G | C | T | T | T | T | T | T | G | A | A | A | G | C | C | A | T | C | C | A | A | G | A | T | T | A | T | G | A | A | G | T | C | C | G | C | 840 |
| RDVER4.SEQ   | G | G | A | G | G | C | T | T | T | C | T | T | G | A | A | A | G | C | C | A | T | C | C | A | A | G | A | T | T | A | T | G | A | A | G | T | C | C | G | C | 840 |
| RDVER5.SEQ   | G | G | A | G | G | C | T | T | T | C | T | T | G | A | A | A | G | C | C | A | T | C | C | A | A | G | A | T | T | A | T | G | A | A | G | T | C | C | G | C | 840 |
| RD7.SEQ      | G | G | A | G | G | C | T | T | T | C | T | T | G | A | A | A | G | C | C | A | T | C | C | A | A | G | A | T | T | A | T | G | A | A | G | T | C | C | G | C | 840 |
| RDVER51.SEQ  | G | G | A | G | G | C | T | T | T | C | T | T | G | A | A | A | G | C | C | A | T | C | C | A | A | G | A | T | T | A | T | G | A | A | G | T | C | C | G | C | 840 |
| RDVER52.SEQ  | G | G | A | G | G | C | T | T | T | C | T | T | G | A | A | A | G | C | C | A | T | C | C | A | A | G | A | T | T | A | T | G | A | A | G | T | C | C | G | C | 840 |
| RD1561H9.SEQ | G | G | A | G | G | C | T | T | T | C | T | T | G | A | A | A | G | C | C | A | T | C | C | A | A | G | A | T | T | A | T | G | A | A | G | T | C | C | G | C | 840 |

Figure 2 (cont.)

GRVER51.SEQ T C C G T G A T C A A C G T C C C T T C A G T C A T T T T G T T C C T G A G C A 880  
GR6.SEQ T C C G T G A T C A A C G T C C C T T C A G T C A T T T T G T T C C T G A G C A 880  
GRVER5.SEQ T C C G T G A T C A A C G T C C C T T C A G T C A T T T T G T T C C T G A G C A 880  
GRVER4.SEQ T C T G T C A T C A A T G T C C C T T C A G T C A T T T T G T T C C T G A G C A 880  
GRVER3.SEQ T C T G T G A T C A A T G T C C C A T C T G T C A T T T T G T T C C T G A G C A 880  
GRVER2.SEQ A G C G T G A T C A A C G T C C C T T C T G T G A T T T T G T T C C T G A G C A 880  
GRVER1.SEQ A G C G T G A T C A A C G T C C C T T C T G T G A T T T T G T T C C T G A G C A 880  
YG81-6G1.SEQ A G T G T A A T T A A C G T T C C A T C A G T A A T A T T G T T C T T A T C G A 880  
RDVER1.SEQ T C T G T C A T T A A T G T G C C A A G C G T C A T C C T G T T T T T G T C T A 880  
RDVER2.SEQ T C T G T C A T T A A T G T G C C A A G C G T C A T C C T G T T T T T G T C T A 880  
RDVER3.SEQ A G C G T C A T T A A C G T G C C T A G C G T G A T C C T G T T T T T G T C T A 880  
RDVER4.SEQ A G T G T C A T C A A C G T G C C T A G C G T G A T C C T G T T T T T G T C T A 880  
RDVER5.SEQ A G T G T C A T C A A C G T G C C T A G C G T G A T C C T G T T T T T G T C T A 880  
RD7.SEQ A G T G T C A T C A A C G T G C C T A G C G T G A T C C T G T T T T T G T C T A 880  
RDVER51.SEQ A G T G T C A T C A A C G T G C C T A G C G T G A T C C T G T T T T T G T C T A 880  
RDVER52.SEQ A G T G T C A T C A A C G T G C C T A G C G T G A T C C T G T T T T T G T C T A 880  
RD1561H9.SEQ A G T G T C A T C A A C G T G C C T A G C G T G A T C C T G T T T T T G T C T A 880

GRVER51.SEQ A A T C T C C T T T G G T T G A C A A G T A T G A T C T G A G C A G C T T G C G 920  
GR6.SEQ A A T C T C C T T T G G T T G A C A A G T A T G A T C T G A G C A G C T T G C G 920  
GRVER5.SEQ A A T C T C C T T T G G T T G A C A A G T A T G A T C T G A G C A G C T T G C G 920  
GRVER4.SEQ A A T C T C C T T T G G T T G A C A A G T A T G A T C T G A G C A G C T T G C G 920  
GRVER3.SEQ A A T C T C C T T T G G T T G A C A A G T A T G A T C T G A G C A G C T T G C G 920  
GRVER2.SEQ A A T C T C C A T T G G T C G A T A A G T A T G A C C T G A G C A G C T T G C G 920  
GRVER1.SEQ A A T C T C C A T T G G T C G A T A A G T A T G A C C T G A G C T C T T T G C G 920  
YG81-6G1.SEQ A A A G T C C T T T G G T T G A C A A A T A C G A T T T A T C A A G T T T A A G 920  
RDVER1.SEQ A G A G C C C T C T G G T G G A C A A A T A C G A T T T G T C T A G C C T G C G 920  
RDVER2.SEQ A G A G C C C T C T G G T G G A C A A A T A C G A T T T G T C T T C T C T G C G 920  
RDVER3.SEQ A G A G C C C A C T C G T G G A C A A G T A C G A C T T G T C T T C C C T G C G 920  
RDVER4.SEQ A G A G C C C A C T C G T G G A C A A G T A C G A C T T G T C T T C A C T G C G 920  
RDVER5.SEQ A G A G C C C A C T C G T G G A C A A G T A C G A C T T G T C T T C A C T G C G 920  
RD7.SEQ A G A G C C C A C T C G T G G A C A A G T A C G A C T T G T C T T C A C T G C G 920  
RDVER51.SEQ A G A G C C C A C T C G T G G A C A A G T A C G A C T T G T C T T C A C T G C G 920  
RDVER52.SEQ A G A G C C C A C T C G T G G A C A A G T A C G A C T T G T C T T C A C T G C G 920  
RD1561H9.SEQ A G A G C C C A C T C G T G G A C A A G T A C G A C T T G T C T T C A C T G C G 920

GRVER51.SEQ T G A G C T G T G C T G T G G C G C T G C T C C T T T G G C C A A A G A A G T G 960  
GR6.SEQ T G A G C T G T G C T G T G G C G C T G C T C C T T T G G C C A A A G A A G T G 960  
GRVER5.SEQ T G A G C T G T G C T G T G G C G C T G C T C C T T T G G C C A A A G A A G T G 960  
GRVER4.SEQ T G A G C T G T G C T G T G G C G C T G C T C C T T T G G C C A A A G A A G T G 960  
GRVER3.SEQ T G A A C T G T G C T G T G G C G C T G C T C C T T T G G C C A A A G A A G T G 960  
GRVER2.SEQ C G A A C T G T G C T G T G G C G C T G C C C C T T T G G C T A A A G A G G T G 960  
GRVER1.SEQ C G A A C T G T G C T G T G G C G C T G C C C C T T T G G C T A A A G A G G T G 960  
YG81-6G1.SEQ G G A A T T G T G T T G C G G T G C G G C A C C A T T A G C A A A G A A G T T 960  
RDVER1.SEQ T G A G T T G T G T T G C G G T G C C G C T C C A C T G G C C A A G G A A G T C 960  
RDVER2.SEQ T G A G T T G T G T T G C G G T G C C G C T C C A C T G G C C A A G G A A G T C 960  
RDVER3.SEQ T G A G T T G T G T T G C G G T G C C G C C C A C T G G C T A A G G A G G T C 960  
RDVER4.SEQ T G A A T T G T G T T G C G G T G C C G C T C C A C T G G C T A A G G A G G T C 960  
RDVER5.SEQ T G A A T T G T G T T G C G G T G C C G C T C C A C T G G C T A A G G A G G T C 960  
RD7.SEQ T G A A T T G T G T T G C G G T G C C G C T C C A C T G G C T A A G G A G G T C 960  
RDVER51.SEQ T G A A T T G T G T T G C G G T G C C G C T C C A C T G G C T A A G G A G G T C 960  
RDVER52.SEQ T G A A T T G T G T T G C G G T G C C G C T C C A C T G G C T A A G G A G G T C 960  
RD1561H9.SEQ T G A A T T G T G T T G C G G T G C C G C T C C A C T G G C T A A G G A G G T C 960



Figure 2 (cont.)

|              |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |      |      |      |
|--------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|------|------|------|
| GRVER51.SEQ  | G | C | C | G | A | G | G | T | C | G | C | T | G | C | T | A | A | G | C | G | T | C | T | G | A | A | C | C | T | C | C | C | T | G | G | T | A | T | C | C    | 1000 |      |
| GR6.SEQ      | G | C | C | G | A | G | G | T | C | G | C | T | G | C | T | A | A | G | C | G | T | C | T | G | A | A | C | C | T | C | C | C | T | G | G | T | A | T | C | C    | 1000 |      |
| GRVER5.SEQ   | G | C | C | G | A | G | G | T | C | G | C | T | G | C | T | A | A | G | C | G | T | C | T | G | A | A | C | C | T | C | C | C | T | G | G | T | A | T | C | C    | 1000 |      |
| GRVER4.SEQ   | G | C | C | G | A | G | G | T | C | G | C | T | G | C | T | A | A | G | C | G | T | C | T | G | A | A | C | C | T | C | C | C | T | G | G | T | A | T | C | C    | 1000 |      |
| GRVER3.SEQ   | G | C | C | G | A | G | G | T | C | G | C | T | G | C | T | A | A | G | C | G | T | C | T | G | A | A | C | C | T | C | C | C | T | G | G | T | A | T | C | C    | 1000 |      |
| GRVER2.SEQ   | G | C | C | G | A | A | G | T | C | G | C | T | G | C | T | C | A | A | G | C | G | T | C | T | G | A | A | T | T | T | G | C | C | A | G | G | T | A | T | C    | C    | 1000 |
| GRVER1.SEQ   | G | C | C | G | A | A | G | T | C | G | C | T | G | C | C | A | A | G | C | G | T | C | T | G | A | A | T | T | T | G | C | C | A | G | G | T | A | T | C | C    | 1000 |      |
| YGB1-6G1.SEQ | G | C | T | G | A | G | G | T | T | G | C | A | G | C | A | A | A | A | C | G | A | T | T | A | A | A | C | T | T | G | C | C | A | G | A | A | T | T | C | 1000 |      |      |
| RDVER1.SEQ   | G | C | T | G | A | G | G | T | G | G | C | C | G | C | T | A | A | A | C | G | C | T | T | G | A | A | C | C | T | G | C | C | T | G | G | C | A | T | T | C    | 1000 |      |
| RDVER2.SEQ   | G | C | T | G | A | G | G | T | G | G | C | C | G | C | T | A | A | A | C | G | C | T | T | G | A | A | C | C | T | G | C | C | T | G | G | C | A | T | T | C    | 1000 |      |
| RDVER3.SEQ   | G | C | T | G | A | A | G | T | G | G | C | C | G | C | C | A | A | A | C | G | C | T | T | G | A | A | T | C | T | G | C | C | A | G | G | C | A | T | T | C    | 1000 |      |
| RDVER4.SEQ   | G | C | T | G | A | A | G | T | G | G | C | C | G | C | C | A | A | A | C | G | C | T | T | G | A | A | T | C | T | G | C | C | C | G | G | C | A | T | T | C    | 1000 |      |
| RDVER5.SEQ   | G | C | T | G | A | A | G | T | G | G | C | C | G | C | C | A | A | A | C | G | C | T | T | G | A | A | T | C | T | T | C | C | A | G | G | G | A | T | T | C    | 1000 |      |
| RD7.SEQ      | G | C | T | G | A | A | G | T | G | G | C | C | G | C | C | A | A | A | C | G | C | T | T | G | A | A | T | C | T | T | C | C | A | G | G | G | A | T | T | C    | 1000 |      |
| RDVER51.SEQ  | G | C | T | G | A | A | G | T | G | G | C | C | G | C | C | A | A | A | C | G | C | T | T | G | A | A | T | C | T | T | C | C | A | G | G | G | A | T | T | C    | 1000 |      |
| RDVER52.SEQ  | G | C | T | G | A | A | G | T | G | G | C | C | G | C | C | A | A | A | C | G | C | T | T | G | A | A | T | C | T | T | C | C | A | G | G | G | A | T | T | C    | 1000 |      |
| RD1561H9.SEQ | G | C | T | G | A | A | G | T | G | G | C | C | G | C | C | A | A | A | C | G | C | T | T | G | A | A | T | C | T | T | C | C | A | G | G | G | A | T | T | C    | 1000 |      |

|              |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |      |      |
|--------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|------|------|
| GRVER51.SEQ  | G | C | T | G | C | G | G | T | T | T | G | G | T | T | T | G | A | C | T | G | A | G | A | G | C | A | C | T | T | C | T | G | C | T | A | A | C | A | T | 1040 |      |
| GR6.SEQ      | G | C | T | G | C | G | G | T | T | T | G | G | T | T | T | G | A | C | T | G | A | G | A | G | C | A | C | T | T | C | T | G | C | T | A | A | C | A | T | 1040 |      |
| GRVER5.SEQ   | G | C | T | G | C | G | G | T | T | T | G | G | T | T | T | G | A | C | T | G | A | G | A | G | C | A | C | T | T | C | T | G | C | T | A | A | C | A | T | 1040 |      |
| GRVER4.SEQ   | G | C | T | G | C | G | G | T | T | T | G | G | T | T | T | G | A | C | T | G | A | G | A | G | C | A | C | T | T | C | T | G | C | T | A | A | C | A | T | 1040 |      |
| GRVER3.SEQ   | G | C | T | G | C | G | G | T | T | T | G | G | T | T | T | G | A | C | T | G | A | G | A | G | C | A | C | T | T | C | T | G | C | C | A | A | C | A | T | 1040 |      |
| GRVER2.SEQ   | G | C | T | G | C | G | G | C | T | T | T | G | G | T | C | T | G | A | C | T | G | A | G | A | G | C | A | C | C | T | C | T | G | C | T | A | A | C | A | T    | 1040 |
| GRVER1.SEQ   | G | C | T | G | C | G | G | C | T | T | T | G | G | T | C | T | G | A | C | T | G | A | G | A | G | C | A | C | C | T | C | T | G | C | T | A | A | C | A | T    | 1040 |
| YG81-6G1.SEQ | G | C | T | G | T | G | G | A | T | T | T | G | G | T | T | T | G | A | C | A | G | A | A | T | C | T | A | C | T | T | C | A | G | C | T | A | A | T | A | T    | 1040 |
| RDVER1.SEQ   | G | T | T | G | T | G | G | T | T | T | C | G | G | C | T | T | G | A | C | C | G | A | A | T | C | T | A | C | T | A | G | C | G | C | C | A | T | T | A | T    | 1040 |
| RDVER2.SEQ   | G | T | T | G | T | G | G | T | T | T | C | G | G | C | T | T | G | A | C | C | G | A | A | T | C | T | A | C | T | A | G | C | G | C | C | A | T | T | A | T    | 1040 |
| RDVER3.SEQ   | G | T | T | G | T | G | G | C | T | T | C | G | G | C | C | T | C | A | C | C | G | A | A | T | C | T | A | C | C | A | G | C | G | C | T | A | T | T | A | T    | 1040 |
| RDVER4.SEQ   | G | T | T | G | T | G | G | C | T | T | C | G | G | C | C | T | C | A | C | C | G | A | A | T | C | T | A | C | C | A | G | C | G | C | T | A | T | T | A | T    | 1040 |
| RDVER5.SEQ   | G | T | T | G | T | G | G | C | T | T | C | G | G | C | C | T | C | A | C | C | G | A | A | T | C | T | A | C | C | A | G | C | G | C | T | A | T | T | A | T    | 1040 |
| RD7.SEQ      | G | T | T | G | T | G | G | C | T | T | C | G | G | C | C | T | C | A | C | C | G | A | A | T | C | T | A | C | C | A | G | C | G | C | T | A | T | T | A | T    | 1040 |
| RDVER51.SEQ  | G | T | T | G | T | G | G | C | T | T | C | G | G | C | C | T | C | A | C | C | G | A | A | T | C | T | A | C | C | A | G | C | G | C | T | A | T | T | A | T    | 1040 |
| RDVER52.SEQ  | G | T | T | G | T | G | G | C | T | T | C | G | G | C | C | T | C | A | C | C | G | A | A | T | C | T | A | C | C | A | G | C | G | C | T | A | T | T | A | T    | 1040 |
| RD1561H9.SEQ | G | T | T | G | T | G | G | C | T | T | C | G | G | C | C | T | C | A | C | C | G | A | A | T | C | T | A | C | C | A | G | T | G | C | G | A | T | T | A | T    | 1040 |

|              |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |      |      |      |      |
|--------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|------|------|------|------|
| GRVER51.SEQ  | C | C | A | T | A | G | C | T | T | G | C | G | A | G | A | C | G | A | G | T | T | T | A | A | G | T | C | T | G | G | T | A | G | C | C | T | G | G | G    | T    | 1080 |      |
| GR6.SEQ      | C | C | A | T | A | G | C | T | T | G | C | G | A | G | A | C | G | A | G | T | T | T | A | A | G | T | C | T | G | G | T | A | G | C | C | T | G | G | G    | T    | 1080 |      |
| GRVER5.SEQ   | C | C | A | T | A | G | C | T | T | G | C | G | A | G | A | C | G | A | G | T | T | T | A | A | G | T | C | T | G | G | T | A | G | C | C | T | G | G | G    | T    | 1080 |      |
| GRVER4.SEQ   | C | C | A | T | A | G | C | T | T | G | C | G | A | G | A | C | G | A | G | T | T | T | A | A | G | T | C | T | G | G | T | A | G | C | C | T | G | G | G    | T    | 1080 |      |
| GRVER3.SEQ   | C | C | A | T | A | G | C | T | T | G | C | G | T | G | A | C | G | A | G | T | T | T | A | A | A | T | C | T | G | G | T | A | G | C | C | T | G | G | G    | T    | 1080 |      |
| GRVER2.SEQ   | T | C | A | T | A | G | C | T | T | G | C | G | T | G | A | T | G | A | G | T | T | C | A | A | A | T | C | T | G | G | T | C | A | G | C | C | T | G | G    | G    | T    | 1080 |
| GRVER1.SEQ   | T | C | A | T | A | G | C | T | T | G | C | G | T | G | A | T | G | A | A | T | T | C | A | A | A | T | C | T | G | G | C | A | G | C | C | T | G | G | G    | T    | 1080 |      |
| YG81-6G1.SEQ | A | C | A | C | A | G | T | C | T | T | A | G | G | A | T | G | A | A | T | T | T | A | A | A | T | C | A | G | A | T | C | A | C | T | T | G | G | A | 1080 |      |      |      |
| RDVER1.SEQ   | C | C | A | A | T | C | T | C | T | G | C | G | C | G | A | C | G | A | G | T | T | T | A | A | G | A | G | C | G | G | T | T | C | T | T | T | G | G | G    | C    | 1080 |      |
| RDVER2.SEQ   | C | C | A | A | T | C | T | C | T | G | C | G | C | G | A | C | G | A | A | T | T | T | A | A | G | A | G | C | G | G | T | T | C | T | T | T | G | G | G    | C    | 1080 |      |
| RDVER3.SEQ   | T | C | A | A | T | C | T | C | T | C | C | G | C | G | A | T | G | A | G | T | T | T | A | A | G | A | G | C | G | G | C | T | C | T | T | T | G | G | G    | C    | 1080 |      |
| RDVER4.SEQ   | T | C | A | G | T | C | T | C | T | C | C | G | C | G | A | T | G | A | G | T | T | T | A | A | G | A | G | C | G | G | C | T | C | T | T | T | G | G | G    | C    | 1080 |      |
| RDVER5.SEQ   | T | C | A | G | T | C | T | C | T | C | C | G | C | G | A | T | G | A | G | T | T | T | A | A | G | A | G | C | G | G | C | T | C | T | T | T | G | G | G    | C    | 1080 |      |
| RD7.SEQ      | T | C | A | G | T | C | T | C | T | C | C | G | C | G | A | T | G | A | G | T | T | T | A | A | G | A | G | C | G | G | C | T | C | T | T | T | G | G | G    | C    | 1080 |      |
| RDVER51.SEQ  | T | C | A | G | T | C | T | C | T | C | C | G | C | G | A | T | G | A | G | T | T | T | A | A | G | A | G | C | G | G | C | T | C | T | T | T | G | G | G    | C    | 1080 |      |
| RDVER52.SEQ  | T | C | A | G | T | C | T | C | T | C | G | G | G | A | T | G | A | G | T | T | T | A | A | G | A | G | C | G | G | C | T | C | T | T | T | G | G | G | C    | 1080 |      |      |
| RD1561H9.SEQ | C | C | A | G | A | C | T | C | T | C | G | G | G | A | T | G | A | G | T | T | T | A | A | G | A | G | C | G | G | C | T | C | T | T | T | G | G | G | C    | 1080 |      |      |

Figure 2 (Cont.)

|              |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |     |   |   |   |   |   |   |   |   |   |   |   |   |      |      |      |      |
|--------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|-----|---|---|---|---|---|---|---|---|---|---|---|---|------|------|------|------|
| GRVER51.SEQ  | C | G | C | G | T | G | A | C | T | C | C | T | C | T | T | A | T | G | G | C | T | G | C | A | A | A   | G | A | T | C | G | C | C | G | A | C | C | G | T    | G    | 1120 |      |
| GR6.SEQ      | C | G | C | G | T | G | A | C | T | C | C | T | C | T | T | A | T | G | G | C | T | G | C | A | A | A   | G | A | T | C | G | C | C | G | A | C | C | G | T    | G    | 1120 |      |
| GRVER5.SEQ   | C | G | C | G | T | G | A | C | T | C | C | T | C | T | T | A | T | G | G | C | T | G | C | A | A | A   | G | A | T | C | G | C | C | G | A | C | C | G | T    | G    | 1120 |      |
| GRVER4.SEQ   | C | G | C | G | T | G | A | C | T | C | C | T | C | T | T | A | T | G | G | C | T | G | C | A | A | A   | G | A | T | C | G | C | C | G | A | C | C | G | T    | G    | 1120 |      |
| GRVER3.SEQ   | C | G | C | G | T | G | A | C | C | C | T | T | T | G | A | T | G | G | C | T | G | C | A | A | A | G   | A | T | C | G | C | C | G | A | C | C | G | T | G    | 1120 |      |      |
| GRVER2.SEQ   | C | G | C | G | T | G | A | C | T | C | C | T | T | T | G | A | T | G | G | C | C | G | C | T | A | A   | G | A | T | C | G | C | C | G | A | C | C | G | T    | G    | 1120 |      |
| GRVER1.SEQ   | C | G | C | G | T | G | A | C | T | C | C | T | T | T | G | A | T | G | G | C | C | G | C | T | A | A   | A | G | A | T | C | G | C | C | G | A | C | C | G    | T    | G    | 1120 |
| YG81-6G1.SEQ | A | G | A | G | T | A | C | T | C | C | T | T | T | A | A | T | G | G | C | A | G | C | T | A | A | A   | A | T | A | G | C | A | G | A | T | A | G | G | 1120 |      |      |      |
| RDVER1.SEQ   | C | G | T | G | T | C | A | C | C | C | A | C | T | G | A | T | G | G | C | T | G | C | C | A | A | A   | A | T | T | G | C | T | G | A | T | C | G | C | G    | 1120 |      |      |
| RDVER2.SEQ   | C | G | T | G | T | C | A | C | C | C | A | C | T | G | A | T | G | G | C | T | G | C | C | A | A | A   | A | T | T | G | C | T | G | A | T | C | G | C | G    | 1120 |      |      |
| RDVER3.SEQ   | C | G | T | G | T | C | A | C | T | C | C | A | C | T | C | A | T | G | G | C | T | G | C | T | A | A   | A | A | T | C | G | C | T | G | A | T | C | G | C    | G    | 1120 |      |
| RDVER4.SEQ   | C | G | T | G | T | C | A | C | T | C | C | A | C | T | C | A | T | G | G | C | T | G | C | T | A | A   | A | G | A | T | C | G | C | T | G | A | T | C | G    | C    | G    | 1120 |
| RDVER5.SEQ   | C | G | T | G | T | C | A | C | T | C | C | A | C | T | C | A | T | G | G | C | T | G | C | T | A | A   | A | G | A | T | C | G | C | T | G | A | T | C | G    | C    | G    | 1120 |
| RD7.SEQ      | C | G | T | G | T | C | A | C | T | C | C | A | C | T | C | A | T | G | G | C | T | G | C | T | A | A   | A | G | A | T | C | G | C | T | G | A | T | C | G    | C    | G    | 1120 |
| RDVER51.SEQ  | C | G | T | G | T | C | A | C | T | C | C | A | C | T | C | A | T | G | G | C | T | G | C | T | A | A   | A | G | A | T | C | G | C | T | G | A | T | C | G    | C    | G    | 1120 |
| RDVER52.SEQ  | C | G | T | G | T | C | A | C | T | C | C | A | C | T | C | A | T | G | G | C | T | G | C | T | A | A   | A | G | A | T | C | G | C | T | G | A | T | C | G    | C    | G    | 1120 |
| RD1561H9.SEQ | C | G | T | G | T | C | A | C | T | C | C | A | C | T | C | A | T | G | G | C | T | G | C | T | A | A   | A | G | A | T | C | G | C | T | G | A | T | C | G    | C    | G    | 1120 |
| GRVER51.SEQ  | A | G | A | C | C | G | G | C | A | A | A | G | C | A | C | T | G | G | G | C | C | C | A | A | A | T   | C | A | A | G | T | C | G | G | T | G | A | A | T    | T    | 1160 |      |
| GR6.SEQ      | A | G | A | C | C | G | G | C | A | A | A | G | C | A | C | T | G | G | G | C | C | C | A | A | A | T   | C | A | A | G | T | C | G | G | T | G | A | A | T    | T    | 1160 |      |
| GRVER5.SEQ   | A | G | A | C | C | G | G | C | A | A | A | G | C | A | C | T | G | G | G | C | C | C | A | A | A | T   | C | A | A | G | T | C | G | G | T | G | A | A | T    | T    | 1160 |      |
| GRVER4.SEQ   | A | G | A | C | C | G | G | C | A | A | A | G | C | A | C | T | G | G | G | C | C | C | A | A | A | T   | C | A | A | G | T | C | G | G | T | G | A | A | T    | T    | 1160 |      |
| GRVER3.SEQ   | A | G | A | C | C | G | G | C | A | A | A | G | C | C | C | T | G | G | G | C | C | C | A | A | A | T   | C | A | G | T | C | G | G | T | G | A | A | T | T    | 1160 |      |      |
| GRVER2.SEQ   | A | G | A | C | C | G | G | C | A | A | A | G | C | T | C | T | G | G | G | T | C | C | A | A | A | T   | C | A | A | G | T | C | G | G | C | G | A | A | T    | T    | 1160 |      |
| GRVER1.SEQ   | A | G | A | C | C | G | G | C | A | A | A | G | C | T | C | T | G | G | G | T | C | C | A | A | A | T   | C | A | A | G | T | C | G | G | C | G | A | A | T    | T    | 1160 |      |
| YG81-6G1.SEQ | A | A | A | C | T | G | G | T | A | A | A | G | C | A | T | T | G | G | G | A | C | C | A | A | A | T   | C | A | A | G | T | T | G | G | T | G | A | A | T    | T    | 1160 |      |
| RDVER1.SEQ   | A | A | A | C | T | G | G | T | A | A | A | G | C | C | T | T | G | G | G | C | C | C | T | A | A | C   | C | A | G | T | T | G | G | T | G | A | A | T | T    | 1160 |      |      |
| RDVER2.SEQ   | A | A | A | C | T | G | G | T | A | A | A | G | C | C | T | T | G | G | G | C | C | C | T | A | A | C   | C | A | G | T | T | G | G | T | G | A | A | T | T    | 1160 |      |      |
| RDVER3.SEQ   | A | A | A | C | T | G | G | T | A | A | A | G | C | T | T | T | G | G | G | C | C | C | T | A | A | C   | C | A | A | G | T | T | G | G | G | C | G | A | G    | C    | T    | 1160 |
| RDVER4.SEQ   | A | A | A | C | T | G | G | T | A | A | A | G | C | T | T | T | G | G | G | C | C | C | T | A | A | C   | C | A | A | G | T | T | G | G | G | C | G | A | G    | C    | T    | 1160 |
| RDVER5.SEQ   | A | A | A | C | T | G | G | T | A | A | A | G | C | T | T | T | G | G | G | C | C | C | T | A | A | C   | C | A | A | G | T | T | G | G | G | C | G | A | G    | C    | T    | 1160 |
| RD7.SEQ      | A | A | A | C | T | G | G | T | A | A | A | G | C | T | T | T | G | G | G | C | C | C | G | A | A | C   | C | A | A | G | T | T | G | G | G | C | G | A | G    | C    | T    | 1160 |
| RDVER51.SEQ  | A | A | A | C | T | G | G | T | A | A | A | G | C | T | T | T | G | G | G | C | C | C | G | A | A | C   | C | A | A | G | T | T | G | G | G | C | G | A | G    | C    | T    | 1160 |
| RDVER52.SEQ  | A | A | A | C | T | G | G | T | A | A | A | G | C | T | T | T | G | G | G | C | C | C | G | A | A | C   | C | A | A | G | T | T | G | G | G | C | G | A | G    | C    | T    | 1160 |
| RD1561H9.SEQ | A | A | A | C | T | G | G | T | A | A | A | G | C | T | T | T | G | G | G | C | C | C | G | A | A | C   | C | A | A | G | T | T | G | G | G | C | G | A | G    | C    | T    | 1160 |
| GRVER51.SEQ  | G | T | G | T | A | T | T | A | A | G | G | G | C | C | C | T | A | T | G | G | T | C | T | C | T | A   | A | A | G | G | C | T | A | C | G | T | G | A | A    | C    | 1200 |      |
| GR6.SEQ      | G | T | G | T | A | T | T | A | A | G | G | G | C | C | C | T | A | T | G | G | T | C | T | C | T | A   | A | A | G | G | C | T | A | C | G | T | G | A | A    | C    | 1200 |      |
| GRVER5.SEQ   | G | T | G | T | A | T | T | A | A | G | G | G | C | C | C | T | A | T | G | G | T | C | T | C | T | A   | A | A | G | G | C | T | A | C | G | T | G | A | A    | C    | 1200 |      |
| GRVER4.SEQ   | G | T | G | T | A | T | T | A | A | G | G | G | C | C | C | T | A | T | G | G | T | C | T | C | T | A   | A | A | G | G | C | T | A | C | G | T | G | A | A    | C    | 1200 |      |
| GRVER3.SEQ   | G | T | G | C | A | T | T | A | A | G | G | G | C | C | C | T | A | T | G | G | T | C | T | C | T | A   | A | A | G | G | C | T | A | C | G | T | G | A | A    | C    | 1200 |      |
| GRVER2.SEQ   | G | T | G | T | A | T | T | A | A | G | G | T | C | C | C | T | A | T | G | G | T | G | T | C | T | A   | A | A | G | G | C | T | A | C | G | T | C | A | A    | C    | 1200 |      |
| GRVER1.SEQ   | G | T | G | T | A | T | T | A | A | G | G | T | C | C | C | T | A | T | G | G | T | G | T | C | T | A   | A | A | G | G | C | T | A | C | G | T | C | A | A    | C    | 1200 |      |
| YG81-6G1.SEQ | A | T | G | C | A | T | T | A | A | A | G | G | T | C | C | C | A | T | G | G | T | A | T | C | G | A   | A | A | G | G | T | T | A | C | G | T | G | A | A    | C    | 1200 |      |
| RDVER1.SEQ   | G | T | G | C | A | T | C | A | A | A | G | G | C | C | C | A | A | T | G | G | T | C | A | G | C | A   | A | G | G | G | T | T | A | T | G | T | G | A | A    | T    | 1200 |      |
| RDVER2.SEQ   | G | T | G | C | A | T | C | A | A | A | G | G | C | C | C | A | A | T | G | G | T | C | A | G | C | A   | A | G | G | G | T | T | A | T | G | T | G | A | A    | T    | 1200 |      |
| RDVER3.SEQ   | G | T | G | T | A | T | C | A | A | A | G | G | C | C | C | T | A | T | G | G | T | G | A | G | C | A   | A | G | G | G | T | T | A | T | G | T | C | A | A    | T    | 1200 |      |
| RDVER4.SEQ   | G | T | G | T | A | T | C | A | A | A | G | G | C | C | C | T | A | T | G | G | T | G | A | G | C | A   | A | G | G | G | T | T | A | T | G | T | C | A | A    | T    | 1200 |      |
| RDVER5.SEQ   | G | T | G | T | A | T | C | A | A | A | G | G | C | C | C | T | A | T | G | G | T | G | A | G | C | A   | A | G | G | G | T | T | A | T | G | T | C | A | A    | T    | 1200 |      |
| RD7.SEQ      | G | T | G | T | A | T | C | A | A | A | G | G | C | C | C | T | A | T | G | G | T | G | A | G | C | A   | A | G | G | G | T | T | A | T | G | T | C | A | A    | T    | 1200 |      |
| RDVER51.SEQ  | G | T | G | T | A | T | C | A | A | A | G | G | C | C | C | T | A | T | G | G | T | G | A | G | C | A   | A | G | G | G | T | T | A | T | G | T | C | A | A    | T    | 1200 |      |
| RDVER52.SEQ  | G | T | G | T | A | T | C | A | A | A | G | G | C | C | C | T | A | T | G | G | T | G | A | G | C | A</ |   |   |   |   |   |   |   |   |   |   |   |   |      |      |      |      |





Figure 2 (cont.)

|              |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |      |      |      |      |
|--------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|------|------|------|------|
| GRVER51.SEQ  | T | A | C | A | A | A | G | G | C | T | C | T | C | A | A | A | G | T | C | G | C | A | C | C | A | G | C | C | G | A | A | C | T | G | G | A | A | G | A | A    | A    | 1360 |      |
| GR6.SEQ      | T | A | C | A | A | A | G | G | C | T | C | T | C | A | A | A | A | G | T | C | G | C | A | C | C | A | G | C | C | G | A | A | C | T | G | G | A | A | G | A    | A    | A    | 1360 |
| GRVER5.SEQ   | T | A | C | A | A | A | G | G | C | T | C | T | C | A | A | A | A | G | T | C | G | C | A | C | C | A | G | C | C | G | A | A | C | T | G | G | A | A | G | A    | A    | A    | 1360 |
| GRVER4.SEQ   | T | A | C | A | A | A | G | G | C | T | C | T | C | A | A | A | A | G | T | C | G | C | C | C | A | G | C | T | G | A | A | C | T | G | G | A | A | G | A | A    | A    | 1360 |      |
| GRVER3.SEQ   | T | A | C | A | A | A | G | G | C | T | C | T | C | A | A | A | A | G | T | C | G | C | C | C | A | G | C | T | G | A | A | C | T | G | G | A | A | G | A | A    | A    | 1360 |      |
| GRVER2.SEQ   | T | A | T | A | A | A | G | G | C | T | C | T | C | A | A | A | A | G | T | C | G | C | C | C | A | G | C | T | G | A | A | G | C | T | G | G | A | A | G | A    | A    | A    | 1360 |
| GRVER1.SEQ   | T | A | T | A | A | A | G | G | C | T | C | T | C | A | A | A | A | G | T | C | G | C | C | C | A | G | C | T | G | A | A | G | C | T | G | G | A | A | G | A    | A    | A    | 1360 |
| YG81-6G1.SEQ | T | A | T | A | A | G | G | G | C | T | C | T | C | A | A | G | T | A | G | C | A | C | C | T | G | C | A | G | A | A | C | T | A | G | A | A | G | A | A | A    | 1360 |      |      |
| RDVER1.SEQ   | T | A | C | A | A | G | G | G | T | A | G | C | C | A | A | A | A | G | T | G | G | C | T | C | C | T | G | C | C | G | A | A | T | T | G | G | A | G | G | A    | A    | A    | 1360 |
| RDVER2.SEQ   | T | A | C | A | A | G | G | G | T | A | G | C | C | A | A | A | A | G | T | G | G | C | T | C | C | T | G | C | C | G | A | A | T | T | G | G | A | G | G | A    | A    | 1360 |      |
| RDVER3.SEQ   | T | A | C | A | A | G | G | G | T | A | G | C | C | A | A | G | T | T | G | C | T | C | C | A | G | C | T | G | A | G | T | T | G | G | A | G | G | A | A | A    | 1360 |      |      |
| RDVER4.SEQ   | T | A | C | A | A | G | G | G | T | A | G | C | C | A | A | G | T | T | G | C | T | C | C | A | G | C | T | G | A | G | T | T | G | G | A | G | G | A | A | A    | 1360 |      |      |
| RDVER5.SEQ   | T | A | C | A | A | G | G | G | T | A | G | C | C | A | A | G | T | T | G | C | T | C | C | A | G | C | T | G | A | G | T | T | G | G | A | G | G | A | A | A    | 1360 |      |      |
| RD7.SEQ      | T | A | C | A | A | G | G | G | T | A | G | C | C | A | A | G | T | T | G | C | T | C | C | A | G | C | T | G | A | G | T | T | G | G | A | G | G | A | A | A    | 1360 |      |      |
| RDVER51.SEQ  | T | A | C | A | A | G | G | G | T | A | G | C | C | A | A | G | T | T | G | C | T | C | C | A | G | C | T | G | A | G | T | T | G | G | A | G | G | A | A | A    | 1360 |      |      |
| RDVER52.SEQ  | T | A | C | A | A | G | G | G | T | A | G | C | C | A | A | G | T | T | G | C | T | C | C | A | G | C | T | G | A | G | T | T | G | G | A | G | G | A | A | A    | 1360 |      |      |
| RD1561H9.SEQ | T | A | C | A | A | G | G | G | T | A | G | C | C | A | A | G | T | T | G | C | T | C | C | A | G | C | T | G | A | G | T | T | G | G | A | G | G | A | A | A    | 1360 |      |      |
|              |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |      |      |      |      |
| GRVER51.SEQ  | T | T | T | T | G | C | T | G | A | A | G | A | A | C | C | C | T | T | G | T | A | T | C | C | G | C | G | A | C | G | T | G | G | C | C | G | T | C | G | T    | 1400 |      |      |
| GR6.SEQ      | T | T | T | T | G | C | T | G | A | A | G | A | A | C | C | C | T | T | G | T | A | T | C | C | G | C | G | A | C | G | T | G | G | C | C | G | T | C | G | T    | 1400 |      |      |
| GRVER5.SEQ   | T | T | T | T | G | C | T | G | A | A | G | A | A | C | C | C | T | T | G | T | A | T | C | C | G | C | G | A | C | G | T | G | G | C | C | G | T | C | G | T    | 1400 |      |      |
| GRVER4.SEQ   | T | T | T | T | G | C | T | G | A | A | G | A | A | C | C | C | T | T | G | T | A | T | C | C | G | C | G | A | C | G | T | G | G | C | C | G | T | C | G | T    | 1400 |      |      |
| GRVER3.SEQ   | T | T | T | T | G | C | T | G | A | A | G | A | A | C | C | C | T | T | G | T | A | T | T | C | G | C | G | A | C | G | T | G | G | C | C | G | T | C | G | T    | 1400 |      |      |
| GRVER2.SEQ   | T | C | T | T | G | C | T | G | A | A | G | A | A | C | C | C | T | T | G | C | A | T | T | C | G | T | G | A | C | G | T | G | G | C | C | G | T | C | G | T    | 1400 |      |      |
| GRVER1.SEQ   | T | C | T | T | G | C | T | G | A | A | G | A | A | C | C | C | T | T | G | C | A | T | T | C | G | T | G | A | C | G | T | G | G | C | C | G | T | C | G | T    | 1400 |      |      |
| YG81-6G1.SEQ | T | T | T | A | T | T | G | A | A | A | A | A | T | C | C | A | T | G | T | A | T | C | A | G | A | G | A | T | G | T | T | G | C | T | G | T | G | G | T | 1400 |      |      |      |
| RDVER1.SEQ   | T | T | C | T | G | T | T | G | A | A | A | A | A | T | C | C | A | T | G | T | A | T | C | C | G | C | G | A | T | G | T | C | G | C | T | G | T | G | G | T    | 1400 |      |      |
| RDVER2.SEQ   | T | T | C | T | G | T | T | G | A | A | A | A | A | T | C | C | A | T | G | T | A | T | C | C | G | C | G | A | T | G | T | C | G | C | T | G | T | G | G | T    | 1400 |      |      |
| RDVER3.SEQ   | T | T | C | T | G | T | T | G | A | A | A | A | A | T | C | C | A | T | G | C | A | T | C | C | G | C | G | A | T | G | T | C | G | C | T | G | T | G | G | T    | 1400 |      |      |
| RDVER4.SEQ   | T | T | C | T | G | T | T | G | A | A | A | A | A | T | C | C | A | T | G | C | A | T | T | C | G | C | G | A | T | G | T | C | G | C | T | G | T | G | G | T    | 1400 |      |      |
| RDVER5.SEQ   | T | T | C | T | G | T | T | G | A | A | A | A | A | T | C | C | A | T | G | C | A | T | T | C | G | C | G | A | T | G | T | C | G | C | T | G | T | G | G | T    | 1400 |      |      |
| RD7.SEQ      | T | T | C | T | G | T | T | G | A | A | A | A | A | T | C | C | A | T | G | C | A | T | T | C | G | C | G | A | T | G | T | C | G | C | T | G | T | G | G | T    | 1400 |      |      |
| RDVER51.SEQ  | T | T | C | T | G | T | T | G | A | A | A | A | A | T | C | C | A | T | G | C | A | T | T | C | G | C | G | A | T | G | T | C | G | C | T | G | T | G | G | T    | 1400 |      |      |
| RDVER52.SEQ  | T | T | C | T | G | T | T | G | A | A | A | A | A | T | C | C | A | T | G | C | A | T | T | C | G | C | G | A | T | G | T | C | G | C | T | G | T | G | G | T    | 1400 |      |      |
| RD1561H9.SEQ | T | T | C | T | G | T | T | G | A | A | A | A | A | T | C | C | A | T | G | C | A | T | T | C | G | C | G | A | T | G | T | C | G | C | T | G | T | G | G | T    | 1400 |      |      |
|              |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |      |      |      |      |
| GRVER51.SEQ  | G | G | G | T | A | T | C | C | C | A | G | A | C | T | T | G | G | A | A | G | C | T | G | G | C | G | A | G | T | T | G | C | C | T | A | G | C | G | C | 1440 |      |      |      |
| GR6.SEQ      | G | G | G | T | A | T | C | C | C | A | G | A | C | T | T | G | G | A | A | G | C | T | G | G | C | G | A | G | T | T | G | C | C | T | A | G | C | G | C | 1440 |      |      |      |
| GRVER5.SEQ   | G | G | G | T | A | T | C | C | C | A | G | A | C | T | T | G | G | A | A | G | C | T | G | G | C | G | A | G | T | T | G | C | C | T | A | G | C | G | C | 1440 |      |      |      |
| GRVER4.SEQ   | G | G | G | T | A | T | C | C | C | A | G | A | C | T | T | G | G | A | A | G | C | T | G | G | T | G | A | G | T | T | G | C | C | T | A | G | C | G | C | 1440 |      |      |      |
| GRVER3.SEQ   | G | G | G | T | A | T | C | C | C | A | G | A | C | T | T | G | G | A | A | G | C | T | G | G | C | G | A | G | T | T | G | C | C | T | A | G | C | G | C | 1440 |      |      |      |
| GRVER2.SEQ   | G | G | G | T | A | T | C | C | C | A | G | A | T | T | T | G | G | A | A | G | C | T | G | G | C | G | A | G | C | T | G | C | C | T | A | G | C | G | C | 1440 |      |      |      |
| GRVER1.SEQ   | G | G | G | T | A | T | C | C | C | A | G | A | T | T | T | G | G | A | A | G | C | T | G | G | C | G | A | G | C | T | G | C | C | T | A | G | C | G | C | 1440 |      |      |      |
| YG81-6G1.SEQ | T | G | G | T | A | T | T | C | C | T | G | A | T | C | T | A | G | A | A | G | C | T | G | G | A | G | A | A | C | T | G | C | C | A | T | C | T | G | C | 1440 |      |      |      |
| RDVER1.SEQ   | C | G | G | C | A | T | T | C | C | T | G | A | C | C | T | G | G | A | G | G | C | C | G | G | T | G | A | A | T | T | G | C | C | A | T | C | T | G | C | 1440 |      |      |      |
| RDVER2.SEQ   | C | G | G | C | A | T | T | C | C | T | G | A | C | C | T | G | G | A | G | G | C | C | G | G | T | G | A | A | T | T | G | C | C | A | T | C | T | G | C | 1440 |      |      |      |
| RDVER3.SEQ   | C | G | G | C | A | T | T | C | C | T | G | A | T | C | T | G | G | A | G | G | C | C | G | G | T | G | A | A | C | T | G | C | C | T | T | C | T | G | C | 1440 |      |      |      |
| RDVER4.SEQ   | C | G | G | C | A | T | T | C | C | T | G | A | T | C | T | G | G | A | G | G | C | C | G | G | C | G | A | A | C | T | G | C | C | T | T | C | T | G | C | 1440 |      |      |      |
| RDVER5.SEQ   | C | G | G | C | A | T | T | C | C | T | G | A | T | C | T | G | G | A | G | G | C | C | G | G | C | G | A | A | C | T | G | C | C | T | T | C | T | G | C | 1440 |      |      |      |
| RD7.SEQ      | C | G | G | C | A | T | T | C | C | T | G | A | T | C | T | G | G | A | G | G | C | C | G | G | C | G | A | A | C | T | G | C | C | T | T | C | T | G | C | 1440 |      |      |      |
| RDVER51.SEQ  | C | G | G | C | A | T | T | C | C | T | G | A | T | C | T | G | G | A | G | G | C | C | G | G | C | G | A | A | C | T | G | C | C | T | T | C | T | G | C | 1440 |      |      |      |
| RDVER52.SEQ  | C | G | G | C | A | T | T | C | C | T | G | A | T | C | T | G | G | A | G | G | C | C | G | G | C | G | A | A | C | T | G | C | C | T | T | C | T | G | C | 1440 |      |      |      |
| RD1561H9.SEQ | C | G | G | C | A | T | T | C | C | T | G | A | T | C | T | G |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |      |      |      |      |

Figure 2 (cont.)

|              |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |      |      |
|--------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|------|------|
| GRVER51.SEQ  | T | T | T | G | T | G | G | T | G | A | A | A | C | A | A | C | C | C | G | G | C | A | A | G | G | A | G | A | T | C | A | C | T | G | C | T | A | A | G | G    | 1480 |
| GR6.SEQ      | T | T | T | G | T | G | G | T | G | A | A | A | C | A | A | C | C | C | G | G | C | A | A | G | G | A | G | A | T | C | A | C | T | G | C | T | A | A | G | G    | 1480 |
| GRVER5.SEQ   | T | T | T | G | T | G | G | T | G | A | A | A | C | A | A | C | C | C | G | G | C | A | A | G | G | A | G | A | T | C | A | C | T | G | C | T | A | A | G | G    | 1480 |
| GRVER4.SEQ   | T | T | T | G | T | G | G | T | G | A | A | A | C | A | A | C | C | T | G | G | C | A | A | G | G | A | G | A | T | C | A | C | T | G | C | T | A | A | G | G    | 1480 |
| GRVER3.SEQ   | T | T | T | G | T | G | G | T | G | A | A | A | C | A | A | C | C | T | G | G | C | A | A | G | G | A | G | A | T | T | A | C | T | G | C | T | A | A | G | G    | 1480 |
| GRVER2.SEQ   | T | T | T | G | T | C | G | T | G | A | A | A | C | A | A | C | C | A | G | G | C | A | A | G | G | A | A | A | T | T | A | C | C | G | C | T | A | A | G | G    | 1480 |
| GRVER1.SEQ   | T | T | T | G | T | C | G | T | G | A | A | A | C | A | A | C | C | A | G | G | T | A | A | G | G | A | A | A | T | T | A | C | C | G | C | T | A | A | G | G    | 1480 |
| YG81-6G1.SEQ | T | T | T | G | T | G | G | T | T | A | A | A | C | A | G | C | C | C | G | G | A | A | G | G | A | G | A | T | T | A | C | A | G | C | T | A | A | G | G | 1480 |      |
| RDVER1.SEQ   | T | T | C | G | T | G | G | T | C | A | A | G | C | A | G | C | C | T | G | G | C | A | A | G | A | G | A | T | C | A | C | T | G | C | C | A | A | G | G | 1480 |      |
| RDVER2.SEQ   | T | T | C | G | T | G | G | T | C | A | A | G | C | A | G | C | C | T | G | G | T | A | A | A | G | A | G | A | T | C | A | C | T | G | C | C | A | A | G | G    | 1480 |
| RDVER3.SEQ   | T | T | C | G | T | C | G | T | C | A | A | G | C | A | G | C | C | T | G | G | T | A | A | A | G | A | A | A | T | C | A | C | C | G | C | C | A | A | G | G    | 1480 |
| RDVER4.SEQ   | T | T | C | G | T | T | G | T | C | A | A | G | C | A | G | C | C | T | G | G | T | A | A | A | G | A | A | A | T | T | A | C | C | G | C | C | A | A | G | G    | 1480 |
| RDVER5.SEQ   | T | T | C | G | T | T | G | T | C | A | A | G | C | A | G | C | C | T | G | G | T | A | A | A | G | A | A | A | T | T | A | C | C | G | C | C | A | A | G | G    | 1480 |
| RD7.SEQ      | T | T | C | G | T | T | G | T | C | A | A | G | C | A | G | C | C | T | G | G | T | A | A | A | G | A | A | A | T | T | A | C | C | G | C | C | A | A | G | G    | 1480 |
| RDVER51.SEQ  | T | T | C | G | T | T | G | T | C | A | A | G | C | A | G | C | C | T | G | G | T | A | A | A | G | A | A | A | T | T | A | C | C | G | C | C | A | A | G | G    | 1480 |
| RDVER52.SEQ  | T | T | C | G | T | T | G | T | C | A | A | G | C | A | G | C | C | T | G | G | T | A | A | A | G | A | A | A | T | T | A | C | C | G | C | C | A | A | G | G    | 1480 |
| RD1561H9.SEQ | T | T | C | G | T | T | G | T | C | A | A | G | C | A | G | C | C | T | G | G | T | A | C | A | G | A | A | A | T | T | A | C | C | G | C | C | A | A | G | G    | 1480 |

|              |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |      |      |      |
|--------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|------|------|------|
| GRVER51.SEQ  | A | G | G | T | C | T | A | C | G | A | C | T | A | T | T | T | G | G | C | C | G | A | G | C | G | C | G | T | G | T | C | T | C | A | C | A | C | C | A    | A    | 1520 |
| GR6.SEQ      | A | G | G | T | C | T | A | C | G | A | C | T | A | T | T | T | G | G | C | C | G | A | G | C | G | C | G | T | G | T | C | T | C | A | C | A | C | C | A    | A    | 1520 |
| GRVER5.SEQ   | A | G | G | T | C | T | A | C | G | A | C | T | A | T | T | T | G | G | C | C | G | A | G | C | G | C | G | T | G | T | C | T | C | A | C | A | C | C | A    | A    | 1520 |
| GRVER4.SEQ   | A | G | G | T | C | T | A | C | G | A | C | T | A | T | T | T | G | G | C | C | G | A | G | C | G | C | G | T | G | T | C | T | C | A | C | A | C | C | A    | A    | 1520 |
| GRVER3.SEQ   | A | G | G | T | C | T | A | C | G | A | C | T | A | T | T | T | G | G | C | C | G | A | G | C | G | C | G | T | G | T | C | T | C | A | C | A | C | T | A    | A    | 1520 |
| GRVER2.SEQ   | A | G | G | T | C | T | A | C | G | A | C | T | A | T | T | T | G | G | C | C | G | A | G | C | G | C | G | T | G | T | C | T | C | A | C | A | C | T | A    | A    | 1520 |
| GRVER1.SEQ   | A | G | G | T | C | T | A | C | G | A | C | T | A | T | T | T | G | G | C | C | G | A | C | G | C | G | T | G | T | C | T | C | A | C | A | C | T | A | A    | 1520 |      |
| YG81-6G1.SEQ | A | A | G | T | G | T | A | C | G | A | T | T | A | T | C | T | T | G | C | C | G | A | G | A | G | G | G | T | C | T | C | C | A | T | A | C | A | A | 1520 |      |      |
| RDVER1.SEQ   | A | A | G | T | G | T | A | T | G | A | T | T | A | C | C | T | G | G | C | T | G | A | C | G | T | G | T | C | A | G | C | C | A | T | A | C | C | A | A    | 1520 |      |
| RDVER2.SEQ   | A | A | G | T | G | T | A | T | G | A | T | T | A | C | C | T | G | G | C | T | G | A | C | G | T | G | T | C | A | G | C | C | A | T | A | C | C | A | A    | 1520 |      |
| RDVER3.SEQ   | A | A | G | T | G | T | A | T | G | A | T | T | A | C | C | T | G | G | C | T | G | A | A | C | G | T | G | T | G | A | G | C | C | A | T | A | C | C | A    | A    | 1520 |
| RDVER4.SEQ   | A | A | G | T | G | T | A | T | G | A | T | T | A | C | C | T | G | G | C | T | G | A | A | C | G | T | G | T | G | A | G | C | C | A | T | A | C | T | A    | A    | 1520 |
| RDVER5.SEQ   | A | A | G | T | G | T | A | T | G | A | T | T | A | C | C | T | G | G | C | T | G | A | A | C | G | T | G | T | G | A | G | C | C | A | T | A | C | T | A    | A    | 1520 |
| RD7.SEQ      | A | A | G | T | G | T | A | T | G | A | T | T | A | C | C | T | G | G | C | T | G | A | A | C | G | T | G | T | G | A | G | C | C | A | T | A | C | T | A    | A    | 1520 |
| RDVER51.SEQ  | A | A | G | T | G | T | A | T | G | A | T | T | A | C | C | T | G | G | C | T | G | A | A | C | G | T | G | T | G | A | G | C | C | A | T | A | C | T | A    | A    | 1520 |
| RDVER52.SEQ  | A | A | G | T | G | T | A | T | G | A | T | T | A | C | C | T | G | G | C | T | G | A | A | C | G | T | G | T | G | A | G | C | C | A | T | A | C | T | A    | A    | 1520 |
| RD1561H9.SEQ | A | A | G | T | G | T | A | T | G | A | T | T | A | C | C | T | G | G | C | T | G | A | A | C | G | T | G | T | G | A | G | C | C | A | T | A | C | T | A    | A    | 1520 |

|              |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |      |      |
|--------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|------|------|
| GRVER51.SEQ  | A | T | A | T | C | T | G | C | G | T | G | G | C | G | G | C | G | T | C | C | G | C | T | T | C | G | T | C | G | A | T | T | C | T | A | T | T | C | C | A    | 1560 |
| GR6.SEQ      | A | T | A | T | C | T | G | C | G | T | G | G | C | G | G | C | G | T | C | C | G | C | T | T | C | G | T | C | G | A | T | T | C | T | A | T | T | C | C | A    | 1560 |
| GRVER5.SEQ   | A | T | A | T | C | T | G | C | G | T | G | G | C | G | G | C | G | T | C | C | G | C | T | T | C | G | T | C | G | A | T | T | C | T | A | T | T | C | C | A    | 1560 |
| GRVER4.SEQ   | A | T | A | T | C | T | G | C | G | T | G | G | C | G | G | C | G | T | C | C | G | C | T | T | C | G | T | C | G | A | T | T | C | C | A | T | C | C | A | 1560 |      |
| GRVER3.SEQ   | A | T | A | T | C | T | G | C | G | T | G | G | C | G | G | C | G | T | C | C | G | C | T | T | C | G | T | C | G | A | T | T | C | T | A | T | C | C | T | 1560 |      |
| GRVER2.SEQ   | G | T | A | C | T | G | C | G | T | G | G | C | G | G | T | G | T | C | C | G | C | T | T | C | G | T | C | G | A | T | A | G | C | A | T | C | C | C | T | 1560 |      |
| GRVER1.SEQ   | G | T | A | C | C | T | G | C | G | T | G | G | C | G | G | T | G | T | C | C | G | C | T | T | C | G | T | G | G | A | T | A | G | C | A | T | C | C | C | T    | 1560 |
| YG81-6G1.SEQ | G | T | A | T | T | T | G | C | G | T | G | G | A | G | G | G | T | T | C | G | A | T | T | C | G | T | T | G | A | T | A | G | C | A | T | A | C | C | A | 1560 |      |
| RDVER1.SEQ   | A | T | A | T | T | T | G | C | G | C | G | G | T | G | G | C | G | T | G | C | G | T | T | T | T | G | T | C | G | A | C | T | C | T | A | T | T | C | C | A    | 1560 |
| RDVER2.SEQ   | A | T | A | T | T | T | G | C | G | C | G | G | T | G | G | C | G | T | G | C | G | T | T | T | T | G | T | G | G | A | C | T | C | T | A | T | T | C | C | A    | 1560 |
| RDVER3.SEQ   | G | T | A | C | T | T | G | C | G | T | G | G | C | G | G | C | G | T | G | C | G | T | T | T | T | G | T | G | G | A | C | A | G | C | A | T | T | C | C | A    | 1560 |
| RDVER4.SEQ   | G | T | A | C | T | T | G | C | G | T | G | G | C | G | G | C | G | T | G | C | G | T | T | T | T | G | T | G | G | A | T | A | G | C | A | T | T | C | C | T    | 1560 |
| RDVER5.SEQ   | G | T | A | C | T | T | G | C | G | T | G | G | C | G | G | C | G | T | G | C | G | T | T | T | T | G | T | T | G | A | C | T | C | C | A | T | C | C | C | T    | 1560 |
| RD7.SEQ      | G | T | A | C | T | T | G | C | G | T | G | G | C | G | G | C | G | T | G | C | G | T | T | T | T | G | T | T | G | A | C | T | C | C | A | T | C | C | C | T    | 1560 |
| RDVER51.SEQ  | G | T | A | C | T | T | G | C | G | T | G | G | C | G | G | C | G | T | G | C | G | T | T | T | T | G | T | T | G | A | C | T | C | C | A | T | C | C | C | T    | 1560 |
| RDVER52.SEQ  | G | T | A | C | T | T | G | C | G | T | G | G | C | G | G | C | G | T | G | C | G | T | T | T | T | G | T | T | G | A | C | T | C | C | A | T | C | C | C | T    | 1560 |
| RD1561H9.SEQ | G | T | A | C | T | T | G | C | G | T | G | G | C | G | G | C | G | T | G | C | G | T | T | T | T | G | T | T | G | A | C | T | C | C | A | T | C | C | C | T    | 1560 |

Figure 2 (cont.)

|              |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |      |      |   |   |   |   |   |      |
|--------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|------|------|---|---|---|---|---|------|
| GRVER51.SEQ  | C | G | C | A | A | C | G | T | T | A | C | C | G | G | T | A | A | G | A | T | C | A | C | T | C | G | T | A | A | A | G | A | G | T | T | G | C | T | G | A    | 1600 |   |   |   |   |   |      |
| GR6.SEQ      | C | G | C | A | A | C | G | T | T | A | C | C | G | G | T | A | A | G | A | T | C | A | C | T | C | G | T | A | A | A | G | A | G | T | T | G | C | T | G | A    | 1600 |   |   |   |   |   |      |
| GRVER5-.SEQ  | C | G | C | A | - | A | C | G | T | T | A | - | C | G | G | T | A | - | G | A | T | C | A | C | - | T | - | C | G | T | A | A | A | G | - | A | G | T | T | G    | C    | - | T | - | G | A | 1600 |
| GRVER4.SEQ   | C | G | C | A | A | C | G | T | G | A | C | C | G | G | T | A | A | G | A | T | C | A | C | T | C | G | T | A | A | A | G | A | A | T | T | G | C | T | G | A    | 1600 |   |   |   |   |   |      |
| GRVER3.SEQ   | C | G | C | A | A | C | G | T | C | A | C | C | G | G | C | A | A | G | A | T | C | A | C | T | C | G | T | A | A | A | G | A | G | T | T | G | C | T | G | A    | 1600 |   |   |   |   |   |      |
| GRVER2.SEQ   | C | G | C | A | A | T | G | T | C | A | C | C | G | G | C | A | A | A | A | T | T | A | C | T | C | G | T | A | A | G | G | A | G | T | T | G | C | T | G | A    | 1600 |   |   |   |   |   |      |
| GRVER1.SEQ   | C | G | C | A | A | T | G | T | C | A | C | C | G | G | C | A | A | A | T | T | A | C | T | C | G | T | A | A | G | A | A | G | T | T | G | C | T | G | A | 1600 |      |   |   |   |   |   |      |
| YG81-6G1.SEQ | A | G | G | A | A | T | G | T | T | A | C | A | G | G | T | A | A | A | A | T | T | A | C | A | A | G | A | A | A | G | A | A | C | T | T | C | T | G | A | 1600 |      |   |   |   |   |   |      |
| RDVER1.SEQ   | C | G | T | A | A | C | G | T | G | A | C | T | G | G | T | A | A | G | A | T | C | A | C | C | C | G | C | A | A | A | G | A | A | C | T | G | T | T | G | A    | 1600 |   |   |   |   |   |      |
| RDVER2.SEQ   | C | G | T | A | A | C | G | T | G | A | C | T | G | G | T | A | A | G | A | T | C | A | C | C | C | G | C | A | A | A | G | A | A | C | T | G | T | T | G | A    | 1600 |   |   |   |   |   |      |
| RDVER3.SEQ   | C | G | T | A | A | T | G | T | G | A | C | T | G | G | T | A | A | A | A | T | T | A | C | C | C | G | C | A | A | G | G | A | A | C | T | G | T | T | G | A    | 1600 |   |   |   |   |   |      |
| RDVER4.SEQ   | C | G | C | A | A | T | G | T | G | A | C | T | G | G | C | A | A | A | A | T | T | A | C | C | C | G | C | A | A | G | G | A | G | C | T | G | T | T | G | A    | 1600 |   |   |   |   |   |      |
| RDVER5.SEQ   | C | G | T | A | A | C | G | T | A | A | C | A | G | G | C | A | A | A | A | T | T | A | C | C | C | G | C | A | A | G | G | A | G | C | T | G | T | T | G | A    | 1600 |   |   |   |   |   |      |
| RD7.SEQ      | C | G | T | A | A | C | G | T | A | A | C | A | G | G | C | A | A | A | A | T | T | A | C | C | C | G | C | A | A | G | G | A | G | C | T | G | T | T | G | A    | 1600 |   |   |   |   |   |      |
| RDVER51.SEQ  | C | G | T | A | A | C | G | T | A | A | C | A | G | G | C | A | A | A | A | T | T | A | C | C | C | G | C | A | A | G | A | G | C | T | G | T | T | G | A | 1600 |      |   |   |   |   |   |      |
| RDVER52.SEQ  | C | G | T | A | A | C | G | T | A | A | C | A | G | G | C | A | A | A | A | T | T | A | C | C | C | G | C | A | A | G | A | G | C | T | G | T | T | G | A | 1600 |      |   |   |   |   |   |      |
| RD1561H9.SEQ | C | G | T | A | A | C | G | T | A | A | C | A | G | G | C | A | A | A | A | T | T | A | C | C | C | G | C | A | A | G | A | G | C | T | G | T | T | G | A | 1600 |      |   |   |   |   |   |      |

|              |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |      |      |
|--------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|------|------|
| GRVER51.SEQ  | A | G | C | A | A | C | T | C | C | T | C | G | A | A | A | A | A | G | C | T | G | G | C | G | G | C | 1626 |      |
| GR6.SEQ      | A | G | C | A | A | C | T | C | C | T | C | G | A | A | A | A | A | G | C | T | G | G | C | G | G | C | 1626 |      |
| GRVER5.SEQ   | A | G | C | A | A | C | T | C | C | T | C | G | A | A | A | A | A | G | C | T | G | G | C | G | G | C | 1626 |      |
| GRVER4.SEQ   | A | G | C | A | A | C | T | C | C | T | C | G | A | A | A | A | A | G | C | T | G | G | C | G | G | C | 1626 |      |
| GRVER3.SEQ   | A | A | C | A | A | T | T | G | C | T | C | G | A | A | A | A | A | G | C | T | G | G | C | G | G | C | 1626 |      |
| GRVER2.SEQ   | A | A | C | A | G | T | T | G | C | T | G | G | A | A | A | A | A | G | G | C | T | G | G | T | G | G | C    | 1626 |
| GRVER1.SEQ   | A | A | C | A | G | T | T | G | C | T | G | G | A | A | A | A | A | G | G | C | T | G | G | T | G | G | C    | 1626 |
| YG81-6G1.SEQ | A | G | C | A | G | T | T | G | C | T | G | G | A | G | A | A | A | G | G | C | G | G | A | G | G | T | 1626 |      |
| RDVER1.SEQ   | A | G | C | A | A | C | T | G | T | T | G | G | A | G | A | A | A | G | C | C | G | G | C | G | G | T | 1626 |      |
| RDVER2.SEQ   | A | G | C | A | A | C | T | G | T | T | G | G | A | G | A | A | A | G | C | C | G | G | C | G | G | T | 1626 |      |
| RDVER3.SEQ   | A | G | C | A | A | T | T | G | T | T | G | G | A | G | A | A | A | G | G | C | C | G | G | C | G | G | T    | 1626 |
| RDVER4.SEQ   | A | A | C | A | A | T | T | G | T | T | G | G | A | G | A | A | A | G | G | C | C | G | G | C | G | G | T    | 1626 |
| RDVER5.SEQ   | A | A | C | A | A | T | T | G | T | T | G | G | A | G | A | A | A | G | G | C | C | G | G | C | G | G | T    | 1626 |
| RD7.SEQ      | A | A | C | A | A | T | T | G | T | T | G | G | A | G | A | A | A | G | G | C | C | G | G | C | G | G | T    | 1626 |
| RDVER51.SEQ  | A | A | C | A | A | T | T | G | T | T | G | G | A | G | A | A | A | G | G | C | C | G | G | C | G | G | T    | 1626 |
| RDVER52.SEQ  | A | A | C | A | A | T | T | G | T | T | G | G | A | G | A | A | A | G | G | C | C | G | G | C | G | G | T    | 1626 |
| RD1561H9.SEQ | A | A | C | A | A | T | T | G | T | T | G | G | T | G | A | A | A | G | G | C | C | G | G | C | G | G | T    | 1626 |



|              |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |     |
|--------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|-----|
| GRVER51.SEQ  | L | P | K | G | V | M | Q | T | H | Q | N | I | C | V | R | L | I | H | A | L | D | P | R | V | G | T | Q | L | I | P | G | V | T | V | L | V | Y | L | P | F | 718 |
| GR6.SEQ      | L | P | K | G | V | M | Q | T | H | Q | N | I | C | V | R | L | I | H | A | L | D | P | R | V | G | T | Q | L | I | S | G | V | T | V | L | V | Y | L | P | F | 718 |
| GRVER5.SEQ   | L | P | K | G | V | M | Q | T | H | Q | N | I | C | V | R | L | I | H | A | L | D | P | R | V | G | T | Q | L | I | P | G | V | T | V | L | V | Y | L | P | F | 718 |
| GRVER4.SEQ   | L | P | K | G | V | M | Q | T | H | Q | N | I | C | V | R | L | I | H | A | L | D | P | R | V | G | T | Q | L | I | P | G | V | T | V | L | V | Y | L | P | F | 718 |
| GRVER3.SEQ   | L | P | K | G | V | M | Q | T | H | Q | N | I | C | V | R | L | I | H | A | L | D | P | R | V | G | T | Q | L | I | P | G | V | T | V | L | V | Y | L | P | F | 718 |
| GRVER2.SEQ   | L | P | K | G | V | M | Q | T | H | Q | N | I | C | V | R | L | I | H | A | L | D | P | R | V | G | T | Q | L | I | P | G | V | T | V | L | V | Y | L | P | F | 718 |
| GRVER1.SEQ   | L | P | K | G | V | M | Q | T | H | Q | N | I | C | V | R | L | I | H | A | L | D | P | R | V | G | T | Q | L | I | P | G | V | T | V | L | V | Y | L | P | F | 718 |
| YG81-6G1.SEQ | L | P | K | G | V | M | Q | T | H | Q | N | I | C | V | R | L | I | H | A | L | D | P | R | A | G | T | Q | L | I | P | G | V | T | V | L | V | Y | L | P | F | 718 |
| RDVER1.SEQ   | L | P | K | G | V | M | Q | T | H | Q | N | I | C | V | R | L | I | H | A | L | D | P | R | Y | G | T | Q | L | I | P | G | V | T | V | L | V | Y | L | P | F | 718 |
| RDVER2.SEQ   | L | P | K | G | V | M | Q | T | H | Q | N | I | C | V | R | L | I | H | A | L | D | P | R | Y | G | T | Q | L | I | P | G | V | T | V | L | V | Y | L | P | F | 718 |
| RDVER3.SEQ   | L | P | K | G | V | M | Q | T | H | Q | N | I | C | V | R | L | I | H | A | L | D | P | R | Y | G | T | Q | L | I | P | G | V | T | V | L | V | Y | L | P | F | 718 |
| RDVER4.SEQ   | L | P | K | G | V | M | Q | T | H | Q | N | I | C | V | R | L | I | H | A | L | D | P | R | Y | G | T | Q | L | I | P | G | V | T | V | L | V | Y | L | P | F | 718 |
| RDVER5.SEQ   | L | P | K | G | V | M | Q | T | H | Q | N | I | C | V | R | L | I | H | A | L | D | P | R | Y | G | T | Q | L | I | P | G | V | T | V | L | V | Y | L | P | F | 718 |
| RD7.SEQ      | L | P | K | G | V | M | Q | T | H | Q | N | I | C | V | R | L | I | H | A | L | D | P | R | Y | G | T | Q | L | I | P | G | V | T | V | L | V | Y | L | P | F | 718 |
| RDVER51.SEQ  | L | P | K | G | V | M | Q | T | H | Q | N | I | C | V | R | L | I | H | A | L | D | P | R | Y | G | T | Q | L | I | P | G | V | T | V | L | V | Y | L | P | F | 718 |
| RDVER52.SEQ  | L | P | K | G | V | M | Q | T | H | Q | N | I | C | V | R | L | I | H | A | L | D | P | R | Y | G | T | Q | L | I | P | G | V | T | V | L | V | Y | L | P | F | 718 |
| RD1561H9.SEQ | L | P | K | G | V | M | Q | T | H | Q | N | I | C | V | R | L | I | H | A | L | D | P | R | Y | G | T | Q | L | I | P | G | V | T | V | L | V | Y | L | P | F | 718 |

[illegible]







Figure 3 (cont.)

|              |   |      |
|--------------|---|------|
| GRVER51.SEQ  | F V V K Q P G K E I T A K E V Y D Y L A E R V S H T K Y L R G G V R F V D S I P   | 1558 |
| GR6.SEQ      | F V V K Q P G K E I T A K E V Y D Y L A E R V S H T K Y L R G G V R F V D S I P   | 1558 |
| GRVER5.SEQ   | F V V K Q P G K E I T A K E V Y D Y L A E R V S H T K Y L R G G V R F V D S I P   | 1558 |
| GRVER4.SEQ   | F V V K Q P G K E I T A K E V Y D Y L A E R V S H T K Y L R G G V R F V D S I P   | 1558 |
| GRVER3.SEQ   | F V V K Q P G K E I T A K E V Y D Y L A E R V S H T K Y L R G G V R F V D S I P   | 1558 |
| GRVER2.SEQ   | F V V K Q P G K E I T A K E V Y D Y L A E R V S H T K Y L R G G V R F V D S I P   | 1558 |
| GRVER1.SEQ   | F V V K Q P G K E I T A K E V Y D Y L A E R V S H T K Y L R G G V R F V D S I P   | 1558 |
| YG81-6G1.SEQ | F V V K Q P G K E I T A K E V Y D Y L A E R V S H T K Y L R G G V R F V D S I P   | 1558 |
| RDVER1.SEQ   | F V V K Q P G K E I T A K E V Y D Y L A E R V S H T K Y L R G G V R F V D S I P   | 1558 |
| RDVER2.SEQ   | F V V K Q P G K E I T A K E V Y D Y L A E R V S H T K Y L R G G V R F V D S I P   | 1558 |
| RDVER3.SEQ   | F V V K Q P G K E I T A K E V Y D Y L A E R V S H T K Y L R G G V R F V D S I P   | 1558 |
| RDVER4.SEQ   | F V V K Q P G K E I T A K E V Y D Y L A E R V S H T K Y L R G G V R F V D S I P   | 1558 |
| RDVER5.SEQ   | F V V K Q P G K E I T A K E V Y D Y L A E R V S H T K Y L R G G V R F V D S I P   | 1558 |
| RD7.SEQ      | F V V K Q P G K E I T A K E V Y D Y L A E R V S H T K Y L R G G V R F V D S I P   | 1558 |
| RDVER51.SEQ  | F V V K Q P G K E I T A K E V Y D Y L A E R V S H T K Y L R G G V R F V D S I P   | 1558 |
| RDVER52.SEQ  | F V V K Q P G K E I T A K E V Y D Y L A E R V S H T K Y L R G G V R F V D S I P   | 1558 |
| RD1561H9.SEQ | F V V K Q P G <span style="border: 1px solid black;">T</span> E I T A K E V Y D Y L A E R V S H T K Y L R G G V R F V D S I P | 1558 |

|              |   |      |
|--------------|---|------|
| GRVER51.SEQ  | R N V T G K I T R K E L L K Q L L E K A G G   | 1624 |
| GR6.SEQ      | R N V T G K I T R K E L L K Q L L E K A G G   | 1624 |
| GRVER5.SEQ   | R N V T G K I T R K E L L K Q L L E K A G G   | 1624 |
| GRVER4.SEQ   | R N V T G K I T R K E L L K Q L L E K A G G   | 1624 |
| GRVER3.SEQ   | R N V T G K I T R K E L L K Q L L E K A G G   | 1624 |
| GRVER2.SEQ   | R N V T G K I T R K E L L K Q L L E K A G G   | 1624 |
| GRVER1.SEQ   | R N V T G K I T R K E L L K Q L L E K A G G   | 1624 |
| YG81-6G1.SEQ | R N V T G K I T R K E L L K Q L L E K A G G   | 1624 |
| RDVER1.SEQ   | R N V T G K I T R K E L L K Q L L E K A G G   | 1624 |
| RDVER2.SEQ   | R N V T G K I T R K E L L K Q L L E K A G G   | 1624 |
| RDVER3.SEQ   | R N V T G K I T R K E L L K Q L L E K A G G   | 1624 |
| RDVER4.SEQ   | R N V T G K I T R K E L L K Q L L E K A G G   | 1624 |
| RDVER5.SEQ   | R N V T G K I T R K E L L K Q L L E K A G G   | 1624 |
| RD7.SEQ      | R N V T G K I T R K E L L K Q L L E K A G G   | 1624 |
| RDVER51.SEQ  | R N V T G K I T R K E L L K Q L L E K A G G   | 1624 |
| RDVER52.SEQ  | R N V T G K I T R K E L L K Q L L E K A G G   | 1624 |
| RD1561H9.SEQ | R N V T G K I T R K E L L K Q L L <span style="border: 1px solid black;">V</span> K A G G | 1624 |

Figure 4 Codon Usage Analysis<sup>21/65</sup>

per 542 total codons

|         | YG81-6G | ver1 GR | ver1 RD | ver5 GR | ver5 RD | HUM |
|---------|---------|---------|---------|---------|---------|-----|
| CGA     | 7       | 0       | 0       | 2       | 0       | 3   |
| CGC     | 1       | 13      | 13      | 11      | 12      | 6   |
| CGG     | 0       | 0       | 0       | 0       | 0       | 6   |
| CGT     | 5       | 13      | 13      | 13      | 14      | 3   |
| AGA     | 6       | 0       | 0       | 0       | 0       | 5   |
| Arg AGG | 7       | 0       | 0       | 0       | 0       | 6   |
| CTA     | 5       | 0       | 0       | 0       | 0       | 3   |
| CTC     | 4       | 0       | 1       | 12      | 11      | 11  |
| CTG     | 4       | 28      | 27      | 19      | 18      | 23  |
| CTT     | 12      | 0       | 0       | 1       | 1       | 6   |
| TTA     | 17      | 0       | 0       | 0       | 0       | 3   |
| Leu TTG | 13      | 27      | 27      | 23      | 25      | 6   |
| TCA     | 6       | 0       | 0       | 1       | 2       | 5   |
| TCC     | 2       | 0       | 0       | 4       | 2       | 10  |
| TCG     | 7       | 0       | 0       | 0       | 0       | 2   |
| TCT     | 7       | 16      | 15      | 11      | 12      | 7   |
| AGC     | 2       | 15      | 15      | 14      | 12      | 10  |
| Ser AGT | 7       | 0       | 0       | 1       | 2       | 5   |
| ACA     | 10      | 0       | 0       | 0       | 1       | 8   |
| ACC     | 2       | 11      | 11      | 8       | 11      | 12  |
| ACG     | 2       | 0       | 0       | 0       | 0       | 4   |
| Thr ACT | 8       | 11      | 11      | 14      | 10      | 7   |
| CCA     | 9       | 14      | 14      | 9       | 12      | 8   |
| CCC     | 8       | 0       | 0       | 2       | 1       | 11  |
| CCG     | 2       | 0       | 0       | 0       | 0       | 4   |
| Pro CCT | 9       | 14      | 14      | 17      | 15      | 8   |
| GCA     | 14      | 0       | 0       | 5       | 4       | 8   |
| GCC     | 4       | 19      | 18      | 14      | 12      | 16  |
| GCG     | 5       | 0       | 0       | 0       | 0       | 4   |
| Ala GCT | 15      | 18      | 19      | 18      | 21      | 11  |
| GGA     | 18      | 0       | 0       | 1       | 3       | 9   |
| GGC     | 3       | 20      | 19      | 21      | 21      | 14  |
| GGG     | 2       | 0       | 0       | 1       | 1       | 9   |
| Gly GGT | 16      | 19      | 20      | 16      | 14      | 6   |
| GTA     | 13      | 0       | 0       | 1       | 1       | 3   |
| GTC     | 4       | 25      | 24      | 21      | 26      | 9   |
| GTG     | 12      | 25      | 25      | 25      | 17      | 17  |
| Val GTT | 20      | 0       | 0       | 3       | 5       | 6   |
| AAA     | 23      | 17      | 18      | 19      | 13      | 12  |
| Lys AAG | 12      | 18      | 17      | 16      | 22      | 19  |
| AAC     | 6       | 11      | 11      | 13      | 12      | 12  |
| Asn AAT | 16      | 11      | 10      | 9       | 9       | 9   |
| CAA     | 8       | 7       | 8       | 11      | 7       | 6   |
| Gln CAG | 6       | 7       | 7       | 3       | 8       | 18  |
| CAC     | 6       | 7       | 6       | 7       | 4       | 8   |
| His CAT | 7       | 6       | 7       | 6       | 9       | 5   |
| GAA     | 26      | 19      | 19      | 19      | 18      | 15  |
| Glu GAG | 12      | 19      | 19      | 19      | 20      | 22  |
| GAC     | 6       | 13      | 13      | 14      | 12      | 16  |
| Asp GAT | 20      | 13      | 13      | 12      | 14      | 12  |
| TAC     | 8       | 10      | 10      | 12      | 13      | 10  |
| Tyr TAT | 11      | 9       | 10      | 7       | 7       | 7   |
| TGC     | 3       | 6       | 5       | 3       | 4       | 8   |
| Cys TGT | 8       | 5       | 6       | 8       | 7       | 5   |
| TTC     | 11      | 13      | 12      | 15      | 12      | 12  |
| Phe TTT | 14      | 12      | 13      | 10      | 13      | 9   |
| ATA     | 12      | 0       | 0       | 0       | 0       | 3   |
| ATC     | 7       | 19      | 19      | 23      | 20      | 13  |
| Ile ATT | 19      | 19      | 20      | 15      | 19      | 8   |
| Met ATG | 11      | 11      | 11      | 11      | 11      | 12  |
| Trp TGG | 2       | 2       | 2       | 2       | 2       | 7   |

relative codon usage for each aa (\*100)

|         | YG81-6G | ver5 GR | ver5 RD | HUM |
|---------|---------|---------|---------|-----|
| CGA     | 27      | 8       | 0       | 10  |
| CGC     | 4       | 42      | 46      | 21  |
| CGG     | 0       | 0       | 0       | 19  |
| CGT     | 19      | 50      | 54      | 9   |
| AGA     | 23      | 0       | 0       | 19  |
| Arg AGG | 27      | 0       | 0       | 21  |
| CTA     | 9       | 0       | 0       | 6   |
| CTC     | 7       | 22      | 20      | 21  |
| CTG     | 7       | 35      | 33      | 44  |
| CTT     | 22      | 2       | 2       | 11  |
| TTA     | 31      | 0       | 0       | 6   |
| Leu TTG | 24      | 42      | 45      | 11  |
| TCA     | 19      | 3       | 7       | 13  |
| TCC     | 6       | 13      | 7       | 25  |
| TCG     | 23      | 0       | 0       | 6   |
| TCT     | 23      | 35      | 40      | 18  |
| AGC     | 6       | 45      | 40      | 26  |
| Ser AGT | 23      | 3       | 7       | 13  |
| ACA     | 45      | 0       | 5       | 25  |
| ACC     | 9       | 36      | 50      | 40  |
| ACG     | 9       | 0       | 0       | 12  |
| Thr ACT | 36      | 64      | 45      | 22  |
| CCA     | 32      | 32      | 43      | 26  |
| CCC     | 29      | 7       | 4       | 35  |
| CCG     | 7       | 0       | 0       | 12  |
| Pro CCT | 32      | 61      | 54      | 27  |
| GCA     | 37      | 13      | 11      | 19  |
| GCC     | 11      | 37      | 32      | 40  |
| GCG     | 13      | 0       | 0       | 10  |
| Ala GCT | 39      | 47      | 55      | 27  |
| GGA     | 46      | 3       | 8       | 24  |
| GGC     | 8       | 54      | 54      | 36  |
| GGG     | 5       | 3       | 3       | 25  |
| Gly GGT | 41      | 41      | 36      | 16  |
| GTA     | 27      | 2       | 2       | 9   |
| GTC     | 8       | 42      | 53      | 25  |
| GTG     | 24      | 50      | 35      | 48  |
| Val GTT | 41      | 6       | 10      | 16  |
| AAA     | 66      | 54      | 37      | 39  |
| Lys AAG | 34      | 46      | 63      | 61  |
| AAC     | 27      | 59      | 57      | 58  |
| Asn AAT | 73      | 41      | 43      | 43  |
| CAA     | 57      | 79      | 47      | 25  |
| Gln CAG | 43      | 21      | 53      | 76  |
| CAC     | 46      | 54      | 31      | 59  |
| His CAT | 54      | 46      | 69      | 39  |
| GAA     | 68      | 50      | 47      | 39  |
| Glu GAG | 32      | 50      | 53      | 61  |
| GAC     | 23      | 54      | 46      | 56  |
| Asp GAT | 77      | 46      | 54      | 42  |
| TAC     | 42      | 63      | 65      | 60  |
| Tyr TAT | 58      | 37      | 35      | 40  |
| TGC     | 27      | 27      | 36      | 60  |
| Cys TGT | 73      | 73      | 64      | 41  |
| TTC     | 44      | 60      | 48      | 58  |
| Phe TTT | 56      | 40      | 52      | 41  |
| ATA     | 32      | 0       | 0       | 13  |
| ATC     | 18      | 61      | 51      | 55  |
| Ile ATT | 50      | 39      | 49      | 34  |
| Met ATG | 100     | 100     | 100     | 100 |
| Trp TGG | 100     | 100     | 100     | 100 |

## Figure 5A

Codon Usage YG#81-6G01 (yellow-green)

|     |     |    |     |     |    |     |     |    |     |     |    |
|-----|-----|----|-----|-----|----|-----|-----|----|-----|-----|----|
| TTT | Phe | 14 | TCT | Ser | 7  | TAT | Tyr | 11 | TGT | Cys | 8  |
| TTC | Phe | 11 | TCC | Ser | 2  | TAC | Tyr | 8  | TGC | Cys | 3  |
| TTA | Leu | 17 | TCA | Ser | 6  | TAA | *** | 0  | TGA | *** | 0  |
| TTG | Leu | 13 | TCG | Ser | 7  | TAG | *** | 0  | TGG | Trp | 2  |
| CTT | Leu | 12 | CCT | Pro | 9  | CAT | His | 7  | CGT | Arg | 5  |
| CTC | Leu | 4  | CCC | Pro | 8  | CAC | His | 6  | CGC | Arg | 1  |
| CTA | Leu | 5  | CCA | Pro | 9  | CAA | Gln | 8  | CGA | Arg | 7  |
| CTG | Leu | 4  | CCG | Pro | 2  | CAG | Gln | 6  | CGG | Arg | 0  |
| ATT | Ile | 19 | ACT | Thr | 8  | AAT | Asn | 16 | AGT | Ser | 7  |
| ATC | Ile | 7  | ACC | Thr | 2  | AAC | Asn | 6  | AGC | Ser | 2  |
| ATA | Ile | 12 | ACA | Thr | 10 | AAA | Lys | 23 | AGA | Arg | 6  |
| ATG | Met | 11 | ACG | Thr | 2  | AAG | Lys | 12 | AGG | Arg | 7  |
| GTT | Val | 20 | GCT | Ala | 15 | GAT | Asp | 20 | GGT | Gly | 16 |
| GTC | Val | 4  | GCC | Ala | 4  | GAC | Asp | 6  | GGC | Gly | 3  |
| GTA | Val | 13 | GCA | Ala | 14 | GAA | Glu | 26 | GGA | Gly | 18 |
| GTG | Val | 12 | GCG | Ala | 5  | GAG | Glu | 12 | GGG | Gly | 2  |

## Figure 5B

## Codon Usage: GRver1

|     |     |    |     |     |    |     |     |    |     |     |    |
|-----|-----|----|-----|-----|----|-----|-----|----|-----|-----|----|
| TTT | Phe | 12 | TCT | Ser | 16 | TAT | Tyr | 9  | TGT | Cys | 5  |
| TTC | Phe | 13 | TCC | Ser | 0  | TAC | Tyr | 10 | TGC | Cys | 6  |
| TTA | Leu | 0  | TCA | Ser | 0  | TAA | *** | 0  | TGA | *** | 0  |
| TTG | Leu | 27 | TCG | Ser | 0  | TAG | *** | 0  | TGG | Trp | 2  |
| CTT | Leu | 0  | CCT | Pro | 14 | CAT | His | 6  | CGT | Arg | 13 |
| CTC | Leu | 0  | CCC | Pro | 0  | CAC | His | 7  | CGC | Arg | 13 |
| CTA | Leu | 0  | CCA | Pro | 14 | CAA | Gln | 7  | CGA | Arg | 0  |
| CTG | Leu | 28 | CCG | Pro | 0  | CAG | Gln | 7  | CGG | Arg | 0  |
| ATT | Ile | 19 | ACT | Thr | 11 | AAT | Asn | 11 | AGT | Ser | 0  |
| ATC | Ile | 19 | ACC | Thr | 11 | AAC | Asn | 11 | AGC | Ser | 15 |
| ATA | Ile | 0  | ACA | Thr | 0  | AAA | Lys | 17 | AGA | Arg | 0  |
| ATG | Met | 11 | ACG | Thr | 0  | AAG | Lys | 18 | AGG | Arg | 0  |
| GTT | Val | 0  | GCT | Ala | 18 | GAT | Asp | 13 | GGT | Gly | 19 |
| GTC | Val | 25 | GCC | Ala | 19 | GAC | Asp | 13 | GGC | Gly | 20 |
| GTA | Val | 0  | GCA | Ala | 0  | GAA | Glu | 19 | GGA | Gly | 0  |
| GTG | Val | 25 | GCG | Ala | 0  | GAG | Glu | 19 | GGG | Gly | 0  |

## Figure 5C

Codon Usage: RDver1

|     |     |    |     |     |    |     |     |    |     |     |    |
|-----|-----|----|-----|-----|----|-----|-----|----|-----|-----|----|
| TTT | Phe | 13 | TCT | Ser | 15 | TAT | Tyr | 10 | TGT | Cys | 6  |
| TTC | Phe | 12 | TCC | Ser | 0  | TAC | Tyr | 10 | TGC | Cys | 5  |
| TTA | Leu | 0  | TCA | Ser | 0  | TAA | *** | 0  | TGA | *** | 0  |
| TTG | Leu | 27 | TCG | Ser | 0  | TAG | *** | 0  | TGG | Trp | 2  |
| CTT | Leu | 0  | CCT | Pro | 14 | CAT | His | 7  | CGT | Arg | 13 |
| CTC | Leu | 1  | CCC | Pro | 0  | CAC | His | 6  | CGC | Arg | 13 |
| CTA | Leu | 0  | CCA | Pro | 14 | CAA | Gln | 8  | CGA | Arg | 0  |
| CTG | Leu | 27 | CCG | Pro | 0  | CAG | Gln | 7  | CGG | Arg | 0  |
| ATT | Ile | 20 | ACT | Thr | 11 | AAT | Asn | 10 | AGT | Ser | 0  |
| ATC | Ile | 19 | ACC | Thr | 11 | AAC | Asn | 11 | AGC | Ser | 15 |
| ATA | Ile | 0  | ACA | Thr | 0  | AAA | Lys | 18 | AGA | Arg | 0  |
| ATG | Met | 11 | ACG | Thr | 0  | AAG | Lys | 17 | AGG | Arg | 0  |
| GTT | Val | 0  | GCT | Ala | 19 | GAT | Asp | 13 | GGT | Gly | 20 |
| GTC | Val | 24 | GCC | Ala | 18 | GAC | Asp | 13 | GGC | Gly | 19 |
| GTA | Val | 0  | GCA | Ala | 0  | GAA | Glu | 19 | GGA | Gly | 0  |
| GTG | Val | 25 | GCG | Ala | 0  | GAG | Glu | 19 | GGG | Gly | 0  |

## Figure 5D

Codon Usage: Grver2

|     |     |    |     |     |    |     |     |    |     |     |    |
|-----|-----|----|-----|-----|----|-----|-----|----|-----|-----|----|
| TTT | Phe | 12 | TCT | Ser | 15 | TAT | Tyr | 9  | TGT | Cys | 5  |
| TTC | Phe | 13 | TCC | Ser | 0  | TAC | Tyr | 10 | TGC | Cys | 6  |
| TTA | Leu | 0  | TCA | Ser | 0  | TAA | *** | 0  | TGA | *** | 0  |
| TTG | Leu | 27 | TCG | Ser | 0  | TAG | *** | 0  | TGG | Trp | 2  |
| CTT | Leu | 0  | CCT | Pro | 14 | CAT | His | 6  | CGT | Arg | 13 |
| CTC | Leu | 0  | CCC | Pro | 0  | CAC | His | 7  | CGC | Arg | 13 |
| CTA | Leu | 0  | CCA | Pro | 14 | CAA | Gln | 10 | CGA | Arg | 0  |
| CTG | Leu | 28 | CCG | Pro | 0  | CAG | Gln | 4  | CGG | Arg | 0  |
| ATT | Ile | 20 | ACT | Thr | 11 | AAT | Asn | 11 | AGT | Ser | 0  |
| ATC | Ile | 18 | ACC | Thr | 11 | AAC | Asn | 11 | AGC | Ser | 16 |
| ATA | Ile | 0  | ACA | Thr | 0  | AAA | Lys | 16 | AGA | Arg | 0  |
| ATG | Met | 11 | ACG | Thr | 0  | AAG | Lys | 19 | AGG | Arg | 0  |
| GTT | Val | 0  | GCT | Ala | 18 | GAT | Asp | 13 | GGT | Gly | 18 |
| GTC | Val | 28 | GCC | Ala | 19 | GAC | Asp | 13 | GGC | Gly | 21 |
| GTA | Val | 0  | GCA | Ala | 0  | GAA | Glu | 17 | GGA | Gly | 0  |
| GTG | Val | 22 | GCG | Ala | 0  | GAG | Glu | 21 | GGG | Gly | 0  |

## Figure 5E

## Codon Usage: Rdver2

|     |     |    |     |     |    |     |     |    |     |     |    |
|-----|-----|----|-----|-----|----|-----|-----|----|-----|-----|----|
| TTT | Phe | 13 | TCT | Ser | 16 | TAT | Tyr | 10 | TGT | Cys | 6  |
| TTC | Phe | 12 | TCC | Ser | 0  | TAC | Tyr | 10 | TGC | Cys | 5  |
| TTA | Leu | 0  | TCA | Ser | 0  | TAA | *** | 0  | TGA | *** | 0  |
| TTG | Leu | 27 | TCG | Ser | 0  | TAG | *** | 0  | TGG | Trp | 2  |
| CTT | Leu | 0  | CCT | Pro | 15 | CAT | His | 7  | CGT | Arg | 13 |
| CTC | Leu | 1  | CCC | Pro | 0  | CAC | His | 6  | CGC | Arg | 13 |
| CTA | Leu | 0  | CCA | Pro | 13 | CAA | Gln | 8  | CGA | Arg | 0  |
| CTG | Leu | 27 | CCG | Pro | 0  | CAG | Gln | 7  | CGG | Arg | 0  |
| ATT | Ile | 19 | ACT | Thr | 11 | AAT | Asn | 10 | AGT | Ser | 0  |
| ATC | Ile | 20 | ACC | Thr | 11 | AAC | Asn | 11 | AGC | Ser | 14 |
| ATA | Ile | 0  | ACA | Thr | 0  | AAA | Lys | 19 | AGA | Arg | 0  |
| ATG | Met | 11 | ACG | Thr | 0  | AAG | Lys | 16 | AGG | Arg | 0  |
| GTT | Val | 0  | GCT | Ala | 19 | GAT | Asp | 13 | GGT | Gly | 21 |
| GTC | Val | 21 | GCC | Ala | 17 | GAC | Asp | 13 | GGC | Gly | 18 |
| GTA | Val | 0  | GCA | Ala | 1  | GAA | Glu | 21 | GGA | Gly | 0  |
| GTG | Val | 28 | GCG | Ala | 0  | GAG | Glu | 17 | GGG | Gly | 0  |

## Figure 5F

Codon Usage: GRver3

|     |     |    |     |     |    |     |     |    |     |     |    |
|-----|-----|----|-----|-----|----|-----|-----|----|-----|-----|----|
| TTT | Phe | 13 | TCT | Ser | 16 | TAT | Tyr | 9  | TGT | Cys | 7  |
| TTC | Phe | 12 | TCC | Ser | 0  | TAC | Tyr | 10 | TGC | Cys | 4  |
| TTA | Leu | 0  | TCA | Ser | 0  | TAA | *** | 0  | TGA | *** | 0  |
| TTG | Leu | 26 | TCG | Ser | 0  | TAG | *** | 0  | TGG | Trp | 2  |
| CTT | Leu | 0  | CCT | Pro | 18 | CAT | His | 6  | CGT | Arg | 14 |
| CTC | Leu | 5  | CCC | Pro | 0  | CAC | His | 7  | CGC | Arg | 12 |
| CTA | Leu | 0  | CCA | Pro | 10 | CAA | Gln | 9  | CGA | Arg | 0  |
| CTG | Leu | 24 | CCG | Pro | 0  | CAG | Gln | 5  | CGG | Arg | 0  |
| ATT | Ile | 14 | ACT | Thr | 14 | AAT | Asn | 11 | AGT | Ser | 0  |
| ATC | Ile | 24 | ACC | Thr | 8  | AAC | Asn | 11 | AGC | Ser | 15 |
| ATA | Ile | 0  | ACA | Thr | 0  | AAA | Lys | 21 | AGA | Arg | 0  |
| ATG | Met | 11 | ACG | Thr | 0  | AAG | Lys | 14 | AGG | Arg | 0  |
| GTT | Val | 1  | GCT | Ala | 18 | GAT | Asp | 12 | GGT | Gly | 18 |
| GTC | Val | 22 | GCC | Ala | 18 | GAC | Asp | 14 | GGC | Gly | 21 |
| GTA | Val | 0  | GCA | Ala | 1  | GAA | Glu | 20 | GGA | Gly | 0  |
| GTG | Val | 27 | GCG | Ala | 0  | GAG | Glu | 18 | GGG | Gly | 0  |



## Figure 5G

Codon Usage: RDver3

|     |     |    |     |     |    |     |     |    |     |     |    |
|-----|-----|----|-----|-----|----|-----|-----|----|-----|-----|----|
| TTT | Phe | 13 | TCT | Ser | 14 | TAT | Tyr | 7  | TGT | Cys | 6  |
| TTC | Phe | 12 | TCC | Ser | 1  | TAC | Tyr | 13 | TGC | Cys | 5  |
| TTA | Leu | 0  | TCA | Ser | 0  | TAA | *** | 0  | TGA | *** | 0  |
| TTG | Leu | 27 | TCG | Ser | 0  | TAG | *** | 0  | TGG | Trp | 2  |
| CTT | Leu | 0  | CCT | Pro | 16 | CAT | His | 10 | CGT | Arg | 16 |
| CTC | Leu | 6  | CCC | Pro | 0  | CAC | His | 3  | CGC | Arg | 10 |
| CTA | Leu | 0  | CCA | Pro | 12 | CAA | Gln | 8  | CGA | Arg | 0  |
| CTG | Leu | 22 | CCG | Pro | 0  | CAG | Gln | 7  | CGG | Arg | 0  |
| ATT | Ile | 20 | ACT | Thr | 10 | AAT | Asn | 10 | AGT | Ser | 0  |
| ATC | Ile | 19 | ACC | Thr | 12 | AAC | Asn | 11 | AGC | Ser | 15 |
| ATA | Ile | 0  | ACA | Thr | 0  | AAA | Lys | 13 | AGA | Arg | 0  |
| ATG | Met | 11 | ACG | Thr | 0  | AAG | Lys | 22 | AGG | Arg | 0  |
| GTT | Val | 0  | GCT | Ala | 20 | GAT | Asp | 14 | GGT | Gly | 16 |
| GTC | Val | 27 | GCC | Ala | 16 | GAC | Asp | 12 | GGC | Gly | 23 |
| GTA | Val | 0  | GCA | Ala | 1  | GAA | Glu | 18 | GGA | Gly | 0  |
| GTG | Val | 22 | GCG | Ala | 0  | GAG | Glu | 20 | GGG | Gly | 0  |

## Figure 5H

Codon Usage: GRver4

|     |     |    |     |     |    |     |     |    |     |     |    |
|-----|-----|----|-----|-----|----|-----|-----|----|-----|-----|----|
| TTT | Phe | 11 | TCT | Ser | 13 | TAT | Tyr | 7  | TGT | Cys | 8  |
| TTC | Phe | 14 | TCC | Ser | 2  | TAC | Tyr | 12 | TGC | Cys | 3  |
| TTA | Leu | 0  | TCA | Ser | 1  | TAA | *** | 0  | TGA | *** | 0  |
| TTG | Leu | 21 | TCG | Ser | 0  | TAG | *** | 0  | TGG | Trp | 2  |
| CTT | Leu | 1  | CCT | Pro | 18 | CAT | His | 7  | CGT | Arg | 14 |
| CTC | Leu | 11 | CCC | Pro | 0  | CAC | His | 6  | CGC | Arg | 11 |
| CTA | Leu | 0  | CCA | Pro | 10 | CAA | Gln | 11 | CGA | Arg | 1  |
| CTG | Leu | 22 | CCG | Pro | 0  | CAG | Gln | 3  | CGG | Arg | 0  |
| ATT | Ile | 13 | ACT | Thr | 14 | AAT | Asn | 11 | AGT | Ser | 1  |
| ATC | Ile | 25 | ACC | Thr | 8  | AAC | Asn | 11 | AGC | Ser | 14 |
| ATA | Ile | 0  | ACA | Thr | 0  | AAA | Lys | 20 | AGA | Arg | 0  |
| ATG | Met | 11 | ACG | Thr | 0  | AAG | Lys | 15 | AGG | Arg | 0  |
| GTT | Val | 3  | GCT | Ala | 19 | GAT | Asp | 12 | GGT | Gly | 17 |
| GTC | Val | 22 | GCC | Ala | 15 | GAC | Asp | 14 | GGC | Gly | 19 |
| GTA | Val | 0  | GCA | Ala | 3  | GAA | Glu | 20 | GGA | Gly | 3  |
| GTG | Val | 25 | GCG | Ala | 0  | GAG | Glu | 18 | GGG | Gly | 0  |

## Figure 5I

## Codon Usage: RDver4

|     |     |    |     |     |    |     |     |    |     |     |    |
|-----|-----|----|-----|-----|----|-----|-----|----|-----|-----|----|
| TTT | Phe | 13 | TCT | Ser | 11 | TAT | Tyr | 7  | TGT | Cys | 7  |
| TTC | Phe | 12 | TCC | Ser | 2  | TAC | Tyr | 13 | TGC | Cys | 4  |
| TTA | Leu | 0  | TCA | Ser | 2  | TAA | *** | 0  | TGA | *** | 0  |
| TTG | Leu | 28 | TCG | Ser | 0  | TAG | *** | 0  | TGG | Trp | 2  |
| CTT | Leu | 0  | CCT | Pro | 16 | CAT | His | 11 | CGT | Arg | 15 |
| CTC | Leu | 7  | CCC | Pro | 2  | CAC | His | 2  | CGC | Arg | 11 |
| CTA | Leu | 0  | CCA | Pro | 10 | CAA | Gln | 7  | CGA | Arg | 0  |
| CTG | Leu | 20 | CCG | Pro | 0  | CAG | Gln | 8  | CGG | Arg | 0  |
| ATT | Ile | 21 | ACT | Thr | 11 | AAT | Asn | 10 | AGT | Ser | 1  |
| ATC | Ile | 18 | ACC | Thr | 11 | AAC | Asn | 11 | AGC | Ser | 14 |
| ATA | Ile | 0  | ACA | Thr | 0  | AAA | Lys | 13 | AGA | Arg | 0  |
| ATG | Met | 11 | ACG | Thr | 0  | AAG | Lys | 22 | AGG | Arg | 0  |
| GTT | Val | 3  | GCT | Ala | 22 | GAT | Asp | 15 | GGT | Gly | 14 |
| GTC | Val | 27 | GCC | Ala | 11 | GAC | Asp | 11 | GGC | Gly | 21 |
| GTA | Val | 0  | GCA | Ala | 4  | GAA | Glu | 18 | GGA | Gly | 4  |
| GTG | Val | 19 | GCG | Ala | 0  | GAG | Glu | 20 | GGG | Gly | 0  |

## Figure 5J

Codon Usage: GRver5

|     |     |    |     |     |    |     |     |    |     |     |    |
|-----|-----|----|-----|-----|----|-----|-----|----|-----|-----|----|
| TTT | Phe | 10 | TCT | Ser | 11 | TAT | Tyr | 7  | TGT | Cys | 8  |
| TTC | Phe | 15 | TCC | Ser | 4  | TAC | Tyr | 12 | TGC | Cys | 3  |
| TTA | Leu | 0  | TCA | Ser | 1  | TAA | *** | 0  | TGA | *** | 0  |
| TTG | Leu | 23 | TCG | Ser | 0  | TAG | *** | 0  | TGG | Trp | 2  |
| CTT | Leu | 1  | CCT | Pro | 17 | CAT | His | 6  | CGT | Arg | 13 |
| CTC | Leu | 12 | CCC | Pro | 2  | CAC | His | 7  | CGC | Arg | 11 |
| CTA | Leu | 0  | CCA | Pro | 9  | CAA | Gln | 11 | CGA | Arg | 2  |
| CTG | Leu | 19 | CCG | Pro | 0  | CAG | Gln | 3  | CGG | Arg | 0  |
| ATT | Ile | 15 | ACT | Thr | 14 | AAT | Asn | 9  | AGT | Ser | 1  |
| ATC | Ile | 23 | ACC | Thr | 8  | AAC | Asn | 13 | AGC | Ser | 14 |
| ATA | Ile | 0  | ACA | Thr | 0  | AAA | Lys | 19 | AGA | Arg | 0  |
| ATG | Met | 11 | ACG | Thr | 0  | AAG | Lys | 16 | AGG | Arg | 0  |
| GTT | Val | 3  | GCT | Ala | 18 | GAT | Asp | 12 | GGT | Gly | 16 |
| GTC | Val | 21 | GCC | Ala | 14 | GAC | Asp | 14 | GGC | Gly | 21 |
| GTA | Val | 1  | GCA | Ala | 5  | GAA | Glu | 19 | GGA | Gly | 1  |
| GTG | Val | 25 | GCG | Ala | 0  | GAG | Glu | 19 | GGG | Gly | 1  |

## Figure 5K

## Codon Usage: RDver5

|     |     |    |     |     |    |     |     |    |     |     |    |
|-----|-----|----|-----|-----|----|-----|-----|----|-----|-----|----|
| TTT | Phe | 13 | TCT | Ser | 12 | TAT | Tyr | 7  | TGT | Cys | 7  |
| TTC | Phe | 12 | TCC | Ser | 2  | TAC | Tyr | 13 | TGC | Cys | 4  |
| TTA | Leu | 0  | TCA | Ser | 2  | TAA | *** | 0  | TGA | *** | 0  |
| TTG | Leu | 25 | TCG | Ser | 0  | TAG | *** | 0  | TGG | Trp | 2  |
| CTT | Leu | 1  | CCT | Pro | 15 | CAT | His | 9  | CGT | Arg | 14 |
| CTC | Leu | 11 | CCC | Pro | 1  | CAC | His | 4  | CGC | Arg | 12 |
| CTA | Leu | 0  | CCA | Pro | 12 | CAA | Gln | 7  | CGA | Arg | 0  |
| CTG | Leu | 18 | CCG | Pro | 0  | CAG | Gln | 8  | CGG | Arg | 0  |
| ATT | Ile | 19 | ACT | Thr | 10 | AAT | Asn | 9  | AGT | Ser | 2  |
| ATC | Ile | 20 | ACC | Thr | 11 | AAC | Asn | 12 | AGC | Ser | 12 |
| ATA | Ile | 0  | ACA | Thr | 1  | AAA | Lys | 13 | AGA | Arg | 0  |
| ATG | Met | 11 | ACG | Thr | 0  | AAG | Lys | 22 | AGG | Arg | 0  |
| GTT | Val | 5  | GCT | Ala | 21 | GAT | Asp | 14 | GGT | Gly | 14 |
| GTC | Val | 26 | GCC | Ala | 12 | GAC | Asp | 12 | GGC | Gly | 21 |
| GTA | Val | 1  | GCA | Ala | 4  | GAA | Glu | 18 | GGA | Gly | 3  |
| GTG | Val | 17 | GCG | Ala | 0  | GAG | Glu | 20 | GGG | Gly | 1  |

## Figure 6

Synthetic oligos for engineered GR/RD genes  
(All oligos listed 5' to 3')

Coding strand: 5' \_\_\_\_\_ (\_\_\_\_\_) n \_\_\_\_\_ 3'  
Non-coding strand: 3' \_\_\_\_\_ (\_\_\_\_\_) n \_\_\_\_\_ 5'

Oligos with pRAM flanking sequence identical for GR/RD

## 1) coding strand upstream flanking

RAM-C1: ACGCCAGCCCAAGCTTAGGCCTGAGTGGC (SEQ ID NO:35)  
RAM-C2: CTTAATTCTCCCCATCCCCCTGTTGACAATTAATCATCGGCTCG (SEQ ID NO:36)  
RAM-C3: TATAATGTGAGGAATTGCGAGCGGATAACAATTTACACA (SEQ ID NO:37)

## 2) coding strand downstream flanking

RAM-C4: ATGGGATGTTACCTAGACCAATATGAAATATTTGGTAAAT (SEQ ID NO:38)  
RAM-C5: AAATGCTTAATGAATTTCAAAAAAAAAAAAAAGGAATTC (SEQ ID NO:39)  
RAM-C6: GATATCAAGCTTATCGATACCGTCGACCTCGAGGATTATA (SEQ ID NO:40)  
RAM-C7: TAGAAAAAGGCCTCGGCGGCGCTAGTTCAGTCAGTT (SEQ ID NO:41)

## 3) non-coding strand downstream flanking

RAM-N1: AACTGACTGAACTAGCG (SEQ ID NO:42)  
RAM-N2: GCCGCCGAGGCCTTTTTCTATATAATCCTCGAGGTCGACG (SEQ ID NO:43)  
RAM-N3: GTATCGATAAGCTTGATATCGAATTCCTTTTTTTTTTTTTT (SEQ ID NO:44)  
RAM-N3b: AGCTTGATATCGAATTCCTTTTTTTTTTTTTTTTGAAATTC (SEQ ID NO:45)  
RAM-N4: TTGAAATTCATTAAGCATTATTTACCAAATATTTTCATAT (SEQ ID NO:46)  
RAM-N5: TGGTCTAGGTAAACATCCCATCACTAGCTTTTTTTTCTATA (SEQ ID NO:47)

## 4) non-coding strand upstream flanking

RAM-N6: TCGCAATTCCTCACATTATACGAGCCGATGATTAATTGTC (SEQ ID NO:48)  
RAM-N7: AACAGGGGGATGGGGAGAATTAAGGCCACTCAGGCCTAAGCTTGGGCTGGCGT (SEQ ID NO:49)

GRver5 with flanking seq. of pRAM to end of *Sfi* I primers

## 1) Coding strand (Start and stop codons are underlined)

GR-C1: GGAAACAGGATCCCATGATGAAACGCGAAAAGAACGTGAT (SEQ ID NO:50)  
GR-C2: CTACGGCCCGAACCACCTGCATCCACTGGAAGACCTCACC (SEQ ID NO:51)  
GR-C3: GCTGGTGAGATGCTCTTCCGAGCACTGCGTAAACATAGTC (SEQ ID NO:52)  
GR-C4: ACCTCCCTCAAGCACTCGTGGACGTCGTGGGAGACGAGAG (SEQ ID NO:53)  
GR-C5: CCTCTCCTACAAAGAATTTTTCGAAGCTACTGTGCTGTTG (SEQ ID NO:54)  
GR-C6: GCCCAAAGCCTCCATAATTGTGGGTACAAATGAACGATG (SEQ ID NO:55)  
GR-C7: TGGTGAGCATTTGTGCTGAGAATAACACTCGCTTCTTTAT (SEQ ID NO:56)  
GR-C8: TCCTGTAATCGCTGCTTGGTACATCGGCATGATTGTCGCC (SEQ ID NO:57)  
GR-C9: CCTGTGAATGAATCTTACATCCAGATGAGCTGTGTAAGG (SEQ ID NO:58)  
GR-C10: TTATGGGTATTAGCAAACCTCAAATCGTCTTTACTACCAA (SEQ ID NO:59)  
GR-C11: AAACATCTTGAATAAGGTCTTGGGAAGTCCAGTCTCGTACT (SEQ ID NO:60)  
GR-C12: AACTTCATCAAACGCATCATTATTCTGGATACCGTCCGAAA (SEQ ID NO:61)  
GR-C13: ACATCCACGGCTGTGAGAGCCTCCCTAACTTCATCTCTCG (SEQ ID NO:62)  
GR-C14: TTACAGCGATGGTAATATCGCTAATTTCAAGCCCTTGCAT (SEQ ID NO:63)  
GR-C15: TTTGATCCAGTCGAGCAAGTGGCCGCTATTTTGTGCTCCT (SEQ ID NO:64)  
GR-C16: CCGGCACCACTGGTTTGCCTAAAGGTGTCTATGCAGACTCA (SEQ ID NO:65)  
GR-C17: CCAGAATATCTGTGTGCGTTTGTATCCACGCTCTCGACCT (SEQ ID NO:66)  
GR-C18: CGTGTGGGTACTCAATTGATCCCTGGCGTGAAGTGTGCTGG (SEQ ID NO:67)  
GR-C19: TGTATCTGCCTTTCTTTCACGCCTTTGGTTTCTCTATTAC (SEQ ID NO:68)  
GR-C20: CCTGGGCTATTTTCATGGTGGCTTGGTGTGTCATCATGTTT (SEQ ID NO:69)

Figure 6 (Cont.)

|   |                 |
|---|-----------------|
| GR-C21: CGTCGCTTCGACCAAGAAGCCTTCTTGAAGGCTATTCAAG      | (SEQ ID NO:70)  |
| GR-C22: ACTACGAGGTGCGTTCCGTGATCAACGTCCCTTCAGTCAT      | (SEQ ID NO:71)  |
| GR-C23: TTTGTTCTTGAGCAAATCTCCTTTGGTTGACAAGTATGATCTG   | (SEQ ID NO:72)  |
| GR-C24: AGCAGCTTGCGTGAGCTGTGCTGTGGCGCTGCTCCTT         | (SEQ ID NO:73)  |
| GR-C25: TGGCCAAAGAAGTGGCCGAGGTCGCTGCTAAGCGTCTGAA      | (SEQ ID NO:74)  |
| GR-C26: CCTCCCTGGTATCCGCTGCGGTTTTGGTTTGAAGTGAAGC      | (SEQ ID NO:75)  |
| GR-C27: ACTTCTGCTAACATCCATAGCTTGCAGACGAGTTTAAGT       | (SEQ ID NO:76)  |
| GR-C28: CTGGTAGCCTGGGTGCGGTGACTCCTCTTATGGCTGCAAA      | (SEQ ID NO:77)  |
| GR-C29: GATCGCCGACCGTGAGACCGGCAAAGCACTGGGCCCAAAT      | (SEQ ID NO:78)  |
| GR-C30: CAAGTCGGTGAATTGTGTATTAAGGGCCCTATGGTCTCTA      | (SEQ ID NO:79)  |
| GR-C31: AAGGCTACGTGAACAATGTGGAGGCCACTAAAGAAGCCAT      | (SEQ ID NO:80)  |
| GR-C32: TGATGATGATGGCTGGCTCCATAGCGGCGACTTCGGTTAC      | (SEQ ID NO:81)  |
| GR-C33: TATGATGAGGACGAACACTTCTATGTGGTCGATCGCTACA      | (SEQ ID NO:82)  |
| GR-C34: AAGAAATTGATTAAAGTACAAAGGCTCTCAAGTCGCACCAGC    | (SEQ ID NO:83)  |
| GR-C35: CGAACTGGAAGAAATTTTGTCTGAAGAACCCTTGTATCCGC     | (SEQ ID NO:84)  |
| GR-C36: GACGTGGCCGTCGTGGGTATCCAGACTTGGAAGCTGGCG       | (SEQ ID NO:85)  |
| GR-C37: AGTTGCCTAGCGCCTTTGTGGTGAACAACCCGGCAAGGA       | (SEQ ID NO:86)  |
| GR-C38: GATCACTGCTAAGGAGGTCTACGACTATTTGGCCGAGCGC      | (SEQ ID NO:87)  |
| GR-C39: GTGTCTCACACCAAATATCTGCGTGGCGGCGTCCGCTTCG      | (SEQ ID NO:88)  |
| GR-C40: TCGATTCTATTCCACGCAACGTTACCGGTAAGATCACTCG      | (SEQ ID NO:89)  |
| GR-C41: TAAAGAGTTGCTGAAGCAACTCCTCGAAAAAGCTGGCGGC      | (SEQ ID NO:90)  |
| GR-C42: TAGTAAAGTCTTCATGATTATATAGAAAAAAGCTAGTG        | (SEQ ID NO:91)  |
| 2) non-coding strand                                  |                 |
| GR-N1: TAATCATGAAGACTTTACTAGCCGCCAGCTTTTTTCGAGGA      | (SEQ ID NO:92)  |
| GR-N2: GTTGCTTCAGCAACTCTTTACGAGTGATCTTACCGGTAAC       | (SEQ ID NO:93)  |
| GR-N3: GTTGCGTGGAATAGAAATCGACGAAGCGGACGCCGCCACG       | (SEQ ID NO:94)  |
| GR-N4: CAGATATTTGGTGTGAGACACGCGCTCGGCCAAATAGTCGT      | (SEQ ID NO:95)  |
| GR-N5: AGACCTCCTTAGCAGTGATCTCCTTGCCGGTTGTTTCAC        | (SEQ ID NO:96)  |
| GR-N6: CACAAAGCGCTAGGCAACTCGCCAGCTTCCAAGTCTGGG        | (SEQ ID NO:97)  |
| GR-N7: ATACCCACGACGGCCACGTCGCGGATACAAGGGTTCTTCA       | (SEQ ID NO:98)  |
| GR-N8: GCAAAATTTCTTCCAGTTTCGGCTGGTCCGACTTGAGAGCC      | (SEQ ID NO:99)  |
| GR-N9: TTTGTACTTAATCAATTCTTTGTAGCGATCGACCACATAG       | (SEQ ID NO:100) |
| GR-N10: AAGTGTTCTGTCCTCATCATAGTAACCGAAGTCGCCGCTAT     | (SEQ ID NO:101) |
| GR-N11: GGAGCCAGCCATCATCATCAATGGCTTCTTTAGTGGCCTC      | (SEQ ID NO:102) |
| GR-N12: CACATTGTTACGCTAGCCTTTAGAGACCATAGGGCCCTTA      | (SEQ ID NO:103) |
| GR-N13: ATACACAATTACACGACTTGATTTGGGCCAGTGCTTTGC       | (SEQ ID NO:104) |
| GR-N14: CGGTCTCACGGTCGGCGATCTTTGCAGCCATAAGAGGAGT      | (SEQ ID NO:105) |
| GR-N15: CACGCGACCCAGGCTACCAGACTTAAACTCGTCTCGCAAG      | (SEQ ID NO:106) |
| GR-N16: CTATGGATGTTAGCAGAAGTGCTCTCAGTCAAACCAAAC       | (SEQ ID NO:107) |
| GR-N17: CGCAGCGGATACAGGGAGGTTACAGCGCTTAGCAGCGAC       | (SEQ ID NO:108) |
| GR-N18: CTCGGCCACTTCTTTGGCCAAAGGAGCAGCGCCACAGCAC      | (SEQ ID NO:109) |
| GR-N19: AGCTCACGCAAGCTGCTCAGATCATACTTGTCAACCAAAG      | (SEQ ID NO:110) |
| GR-N20: GAGATTTGCTCAGGAACAAATGACTGAAGGGACGTTGAT       | (SEQ ID NO:111) |
| GR-N21: CACGGAACGCACCTCGTAGTCTTGAATAGCCTTCAA          | (SEQ ID NO:112) |
| GR-N22: GAAGGCTTCTTGGTCTGAAGCGACGAAACATGATGACACGCAAGC | (SEQ ID NO:113) |
| GR-N23: CGACCATGAAATAGCCCAGGGTAATAGAGAAACCAAAGGC      | (SEQ ID NO:114) |
| GR-N24: GTGAAAGAAAGGCAGATACACCAGCACAGTCACGCCAGGG      | (SEQ ID NO:115) |
| GR-N25: ATCAATTGAGTACCCACACGAGGGTCGAGAGCGTGGATCA      | (SEQ ID NO:116) |
| GR-N26: AACGCACACAGATATTCTGGTGAGTCTGCATGACACCTTT      | (SEQ ID NO:117) |
| GR-N27: AGGCAAACAGTGGTGCCGGAGGAGCACAAATAGCGGCC        | (SEQ ID NO:118) |

Figure 6 (Cont..)

GR-N28: ACTTGCTCGACTGGATCAAAATGCAAGGGCTTGAAATTAG (SEQ ID NO:119)  
 GR-N29: CGATATTACCATCGCTGTAACGAGAGATGAAGTTAGGGAG (SEQ ID NO:120)  
 GR-N30: GCTCTCACAGCCGTGGATGTTTTTCGACGGTATCCAGAATA (SEQ ID NO:121)  
 GR-N31: ATGATGCGTTTGATGAAGTTAGTACGAGACTGGACTTCCA (SEQ ID NO:122)  
 GR-N32: AGACCTTATTCAAGATGTTTTTGGTAGTAAAGACGATTG (SEQ ID NO:123)  
 GR-N33: AGGTTTGCTAATAACCCATAACCTTACACAGCTCATCTGGG (SEQ ID NO:124)  
 GR-N34: ATGTAAGATTCATTACAGGGGCGACAATCATGCCGATGT (SEQ ID NO:125)  
 GR-N35: ACCAAGCAGCGATTACAGGAATAAAGAAGCGAGTGTTATT (SEQ ID NO:126)  
 GR-N36: CTCAGCACAAATGCTCACCACATCGTTCATTTGTACCCA (SEQ ID NO:127)  
 GR-N37: CAATTATGGAGGCTTTGGGCCAACACAGTAGCTTCGA (SEQ ID NO:128)  
 GR-N38: AAAATTCTTTGTAGGAGAGGCTCTCGTCTCCACGACGTC (SEQ ID NO:129)  
 GR-N39: CAGAGTGCTTGAGGGAGGTGACTATGTTTACGCAGTGCT (SEQ ID NO:130)  
 GR-N40: CGGAAGAGCATCTCACCAGCGGTGAGGTCTTCCAGTGGAT (SEQ ID NO:131)  
 GR-N41: GCAGTGGTTCTGGGCCGTAGATCACGTTCTTTTCGCGTTT (SEQ ID NO:132)  
 GR-N42: CATCATGGGATCCTGTTTCCTGTGTGAAATTGTTATCCGC (SEQ ID NO:133)

RDver5 with flanking sequence of pRAM to end of Sfi I primers

1) coding strand

RD-C1: GGAAACAGGATCCCATGATGAAGCGTGAGAAAAATGTCAT (SEQ ID NO:134)  
 RD-C2: CTATGGCCCTGAGCCTCTCCATCCTTTGGAGGATTTGACT (SEQ ID NO:135)  
 RD-C3: GCCGGCGAAATGCTGTTTCGTGCTCTCCGCAAGCACTCTC (SEQ ID NO:136)  
 RD-C4: ATTTGCCTCAAGCCTTGGTCTGATGTGGTCGGCGATGAATC (SEQ ID NO:137)  
 RD-C5: TTTGAGCTACAAGGAGTTTTTTTGAGGCAACCGTCTTGCTG (SEQ ID NO:138)  
 RD-C6: GCTCAGTCCCTCCACAATTGTGGGTACAAGATGAACGACG (SEQ ID NO:139)  
 RD-C7: TCGTTAGTATCTGTGCTGAAAACAATACCCGTTTCTTCAT (SEQ ID NO:140)  
 RD-C8: TCCAGTCATCGCCGATGGTATATCGGTATGATCGTGGCT (SEQ ID NO:141)  
 RD-C9: CCAGTCAACGAGAGCTACATTCCTCCGACGAAGTGTGTAAAG (SEQ ID NO:142)  
 RD-C10: TCATGGGTATCTCTAAGCCACAGATTGTCTTCACCACTAA (SEQ ID NO:143)  
 RD-C11: GAATATTCTGAACAAAGTCCTGGAAGTCCAAAGCCGCACC (SEQ ID NO:144)  
 RD-C12: AACTTTATTAAGCGTATCATCATCTTGACACTGTGGAGA (SEQ ID NO:145)  
 RD-C13: ATATTCACGGTTGCGAATCTTTGCCCTAATTCATCTCTCG (SEQ ID NO:146)  
 RD-C14: CTATTCAGCAGCGCAACATCGCAACTTTAAACCACTCCAC (SEQ ID NO:147)  
 RD-C15: TTCGACCCTGTGGAACAAGTTGCAGCCATCTGTGTAGCA (SEQ ID NO:148)  
 RD-C16: GCGGTACTACTGGACTCCCAAAGGGAGTCATGCAGACCCA (SEQ ID NO:149)  
 RD-C17: TCAAAACATTTGCGTGCGTCTGATCCATGCTCTCGATCCA (SEQ ID NO:150)  
 RD-C18: CGCTACGGCACTCAGCTGATTCCTGGTGTACCCGTCTTGG (SEQ ID NO:151)  
 RD-C19: TCTACTTGCCTTTCTTCCATGCTTTCGGCTTTCATATTAC (SEQ ID NO:152)  
 RD-C20: TTTGGGTTACTTTATGGTGGTCTCCGCGTGATTATGTTT (SEQ ID NO:153)  
 RD-C21: CGCCGTTTGTATCAGGAGGCTTCTTGAAAGCCATCCAAG (SEQ ID NO:154)  
 RD-C22: ATTATGAAGTCCGCAAGTGTATCAACGTGCCTAGCGTGAT (SEQ ID NO:155)  
 RD-C23: CCTGTTTTTGTCTAAGAGCCCACTCGTGACAAAGTACGAC (SEQ ID NO:156)  
 RD-C24: TTGTCTTCACTGCGTGAATTGTGTTGCGGTGCCGCTCCAC (SEQ ID NO:157)  
 RD-C25: TGGCTAAGGAGGTGCTGAAAGTGGCCGCCAAACGCTTGAA (SEQ ID NO:158)  
 RD-C26: TCTTCCAGGGATTCTGTTGTGGCTTCGGCCTCACCGAATCT (SEQ ID NO:159)  
 RD-C27: ACCAGCGCTATTATTCAGTCTCTCCGCGATGAGTTTAAGA (SEQ ID NO:160)  
 RD-C28: GCGGCTCTTTGGGCCGTGTCACTCCACTCATGGCTGCTAA (SEQ ID NO:161)  
 RD-C29: GATCGCTGATCGCGAAACTGGTAAGGCTTTGGGCCCTAAC (SEQ ID NO:162)  
 RD-C30: CAAGTGGGCGAGCTGTGTATCAAAGGCCCTATGGTGAGCA (SEQ ID NO:163)  
 RD-C31: AGGGTTATGTCAATAACGTCTGAAGCTACCAAGGAGGCCAT (SEQ ID NO:164)  
 RD-C32: CGACGACGACGGCTGGTTGCATTCTGGTGATTTTGGATAT (SEQ ID NO:165)  
 RD-C33: TACGACGAAGATGAGCATTTTTACGTCGTGGATCGTTACA (SEQ ID NO:166)  
 RD-C34: AGGAGCTGATCAAATACAAGGGTAGCCAGGTTGCTCCAGC (SEQ ID NO:167)  
 RD-C35: TGAGTTGGAGGAGATTCTGTTGAAAAATCCATGCATTCCG (SEQ ID NO:168)



Figure 6 (Cont.)

RD-C36: GATGTCGCTGTGGTCGGCATTCCCTGATCTGGAGGCCGGCG (SEQ ID NO:169)  
 RD-C37: AACTGCCTTCTGCTTTTCGTTGTCAAGCAGCCTGGTAAAGA (SEQ ID NO:170)  
 RD-C38: AATTACCGCCAAAGAAGTGTATGATTACCTGGCTGAACGT (SEQ ID NO:171)  
 RD-C39: GTGAGCCATACTAAGTACTTGCGTGGCGGCGTGCCTTTTG (SEQ ID NO:172)  
 RD-C40: TTGACTCCATCCCTCGTAACGTAACAGGCAAAATTACCCG (SEQ ID NO:173)  
 RD-C41: CAAGGAGCTGTTGAAACAATTGTTGGAGAAGGCCGGCGGT (SEQ ID NO:174)  
 RD-C42: TAGTAAAGTCTTCATGATTATATAGAAAAAAGCTAGTG (SEQ ID NO:175)

## 2) non-coding strand

RD-N1: TAATCATGAAGACTTTACTAACCGCCGGCCTTCTCCAACA (SEQ ID NO:176)  
 RD-N2: ATTGTTTCAACAGCTCCTTGCGGGTAATTTTGCCTGTTAC (SEQ ID NO:177)  
 RD-N3: GTTACGAGGGATGGAGTCAACAAAACGCACGCCGCCACGC (SEQ ID NO:178)  
 RD-N4: AAGTACTTAGTATGGCTCACACGTTTCAGCCAGGTAATCAT (SEQ ID NO:179)  
 RD-N5: ACACTTCTTTGGCGGTAATTTCTTTACCAGGCTGCTTGAC (SEQ ID NO:180)  
 RD-N6: AACGAAAGCAGAAGGCAGTTCGCCGGCCTCCAGATCAGGA (SEQ ID NO:181)  
 RD-N7: ATGCCGACCACAGCGACATCGCGAATGCATGGATTTTCA (SEQ ID NO:182)  
 RD-N8: ACAGAATCTCCTCCAACCTCAGCTGGAGCAACCTGGCTACC (SEQ ID NO:183)  
 RD-N9: CTTGTATTTGATCAGCTCCTTGTAACGATCCACGACGTAA (SEQ ID NO:184)  
 RD-N10: AAATGCTCATCTTCGTCGTAATATCCAAATCACCAGAAT (SEQ ID NO:185)  
 RD-N11: GCAACCAGCCGTCGTCGTCGATGGCCTCCTTGGTAGCTTC (SEQ ID NO:186)  
 RD-N12: GACGTTATTGACATAACCCTTGCTCACCATAGGGCCTTTG (SEQ ID NO:187)  
 RD-N13: ATACACAGCTCGCCCACTTGGTTAGGGCCCAAGCCTTAC (SEQ ID NO:188)  
 RD-N14: CAGTTTCGCGATCAGCGATCTTAGCAGCCATGAGTGGAGT (SEQ ID NO:189)  
 RD-N15: GACACGGCCCAAAGAGCCGCTCTTAAACTCATCGCGGAGA (SEQ ID NO:190)  
 RD-N16: GACTGAATAATAGCGCTGGTAGATTTCGGTGAGGCCGA (SEQ ID NO:191)  
 RD-N17: AGCCACAACGAATCCCTGGAAGATTCAAGCGTTTGGCGGCCAC (SEQ ID NO:192)  
 RD-N18: TTCAGCGACCTCCTTAGCCAGTGGAGCGGCACCGCAACAC (SEQ ID NO:193)  
 RD-N19: AATTCACGCAGTGAAGACAAGTCGTACTTGTCCACGAGTG (SEQ ID NO:194)  
 RD-N20: GGCTCTTAGACAAAAACAGGATCAGCTAGGCACGTTGAT (SEQ ID NO:195)  
 RD-N21: GACACTGCGGACTTCATAATCTTGGATGGCTTTCAAGAAA (SEQ ID NO:196)  
 RD-N22: GCCTCCTGATCAAAACGGCGGAACATAATCACGCGGAGAC (SEQ ID NO:197)  
 RD-N23: CGACCATAAAGTAACCCAAAGTAATATGAAAGCCGAAAGC (SEQ ID NO:198)  
 RD-N24: ATGGAAGAAAGGCAAGTAGACCAAGACGGTGACACCAGGA (SEQ ID NO:199)  
 RD-N25: ATCAGCTGAGTGCCGTAGCGTGGATCGAGAGCATGGATCA (SEQ ID NO:200)  
 RD-N26: GACGCACGCAATGTTTGTATGGGTCTGCATGACTCCCTT (SEQ ID NO:201)  
 RD-N27: TGGGAGTCCAGTAGTACCGCTGCTACACAGAATGGCTGCA (SEQ ID NO:202)  
 RD-N28: ACTTGTTCCACAGGGTCGAAGTGGAGTGGTTTAAAGTTTG (SEQ ID NO:203)  
 RD-N29: CGATGTTGCCGTCTGAATAGCGAGAGATGAAATTAGGCAA (SEQ ID NO:204)  
 RD-N30: AGATTGCGAACCGTGAATATTCTCCACAGTGTCCAAGATG (SEQ ID NO:205)  
 RD-N31: ATGATACGCTTAATAAAGTTGGTGCGGCTTTGGACTTCCA (SEQ ID NO:206)  
 RD-N32: GGACTTTGTTTCAGAAATATTCTTAGTGGTGAAGACAATCTG (SEQ ID NO:207)  
 RD-N33: TGGCTTAGAGATACCCATGACTTTACACAGTTTCGTCCGGA (SEQ ID NO:208)  
 RD-N34: ATGTAGCTCTCGTTGACTGGAGCCACGATCATACCGATAT (SEQ ID NO:209)  
 RD-N35: ACCATGCGGCGATGACTGGAATGAAGAAACGGGTATTGTT (SEQ ID NO:210)  
 RD-N36: TTCAGCACAGATACTAACGACGTCGTTTCATCTTGTAGCCA (SEQ ID NO:211)  
 RD-N37: CAATTGTGGAGGGACTGAGCCAGCAAGACGGTTGCCTCAA (SEQ ID NO:212)  
 RD-N38: AAAACTCCTTGTTAGCTCAAAGATTCATCGCCGACCACATC (SEQ ID NO:213)  
 RD-N39: GACCAAGGCTTGAGGCAAATGAGAGTGCTTGCGGAGAGCA (SEQ ID NO:214)  
 RD-N40: CGAAACAGCATTTTCGCCGGCAGTCAAATCCTCCAAAGGAT (SEQ ID NO:215)  
 RD-N41: GGAGAGGCTCAGGGCCATAGATGACATTTTCTCACGCTT (SEQ ID NO:216)  
 RD-N42: CATCATGGGATCCTGTTTCCTGTGTGAAATTGTTATCCGC (SEQ ID NO:217)

Figure 7

RELLUC.SEQ A T G A C T T C G A A A G T T T A T G A T C C A G A A C A A A G G A A A C G G A 40  
RLUCVER1.SEQ A T G G C T T C C A A G G T G T A C G A C C C C G A G C A G C G C A A G C G C A 40  
RLUCVER2.SEQ A T G G C T T C C A A G G T G T A C G A C C C C G A G C A A C G C A A A C G C A 40  
RLUCFINL.SEQ A T G G C T T C C A A G G T G T A C G A C C C C G A G C A A C G C A A A C G C A 40

RELLUC.SEQ T G A T A A C T G G T C C G C A G T G G T G G G C C A G A T G T A A A C A A A T 80  
RLUCVER1.SEQ T G A T C A C C G G C C C T C A G T G G T G G G C C C G C T G C A A G C A G A T 80  
RLUCVER2.SEQ T G A T C A C T G G G C C T C A G T G G T G G G C T C G C T G C A A G C A A A T 80  
RLUCFINL.SEQ T G A T C A C T G G G C C T C A G T G G T G G G C T C G C T G C A A G C A A A T 80

RELLUC.SEQ G A A T G T T C T T G A T T C A T T T A T T A A T T A T T A T G A T T C A G A A 120  
RLUCVER1.SEQ G A A C G T G C T G G A C T C C T T C A T C A A C T A C T A C G A C A G C G A G 120  
RLUCVER2.SEQ G A A C G T G C T G G A C T C C T T C A T C A A C T A C T A T G A T T C C G A G 120  
RLUCFINL.SEQ G A A C G T G C T G G A C T C C T T C A T C A A C T A C T A T G A T T C C G A G 120

RELLUC.SEQ A A A C A T G C A G A A A A T G C T G T T A T T T T T T A C A T G G T A A C G 160  
RLUCVER1.SEQ A A G C A C G C C G A G A A C G C C G T G A T C T T C C T G C A C G G C A A C G 160  
RLUCVER2.SEQ A A G C A C G C C G A G A A C G C C G T G A T T T T T C T G C A T G G T A A C G 160  
RLUCFINL.SEQ A A G C A C G C C G A G A A C G C C G T G A T T T T T C T G C A T G G T A A C G 160

RELLUC.SEQ C G G C C T C T T C T T A T T T A T G G C G A C A T G T T G T G C C A C A T A T 200  
RLUCVER1.SEQ C G C C T C C A G C T A C C T G T G G A G G C A C G T G G T G C C T C A C A T 200  
RLUCVER2.SEQ C T G C C T C C A G C T A C C T G T G G A G G C A C G T C G T G C C T C A C A T 200  
RLUCFINL.SEQ C T G C C T C C A G C T A C C T G T G G A G G C A C G T C G T G C C T C A C A T 200

RELLUC.SEQ T G A G C C A G T A G C G C G G T G T A T T A T A C C A G A T C T T A T T G G T 240  
RLUCVER1.SEQ C G A G C C C G T G G C C C G C T G C A T C A T C C C T G A C C T G A T C G G C 240  
RLUCVER2.SEQ C G A G C C C G T G G C T C G C T G C A T C A T C C C T G A T C T G A T C G G A 240  
RLUCFINL.SEQ C G A G C C C G T G G C T A G A T G C A T C A T C C C T G A T C T G A T C G G A 240

RELLUC.SEQ A T G G G C A A A T C A G G C A A A T C T G G T A A T G G T T C T T A T A G G T 280  
RLUCVER1.SEQ A T G G G C A A G T C C G G C A A G A G C G G C A A C G G C T C C T A C C G C C 280  
RLUCVER2.SEQ A T G G G T A A G T C C G G C A A G A G C G G G A A A T G G C T C A T A T C G C C 280  
RLUCFINL.SEQ A T G G G T A A G T C C G G C A A G A G C G G G A A A T G G C T C A T A T C G C C 280

RELLUC.SEQ T A C T T G A T C A T T A C A A A T A T C T T A C T G C A T G G T T T G A A C T 320  
RLUCVER1.SEQ T G C T G G A C C A C T A C A A G T A C C T G A C C G C C T G G T T C G A G C T 320  
RLUCVER2.SEQ T C C T G G A T C A C T A C A A G T A C C T C A C C G C T T G G T T C G A G C T 320  
RLUCFINL.SEQ T C C T G G A T C A C T A C A A G T A C C T C A C C G C T T G G T T C G A G C T 320

RELLUC.SEQ T C T T A A T T T A C C A A A G A A G A T C A T T T T T G T C G G C C A T G A T 360  
RLUCVER1.SEQ G C T G A A C C T T G C C C A A G A A G A T C A T C T T C G T G G G C C A C G A C 360  
RLUCVER2.SEQ G C T G A A C C T T C C A A A G A A A A T C A T C T T T G T G G G C C A C G A C 360  
RLUCFINL.SEQ G C T G A A C C T T C C A A A G A A A A T C A T C T T T G T G G G C C A C G A C 360

RELLUC.SEQ T G G G G T G C T T G T T T G G C A T T T C A T T A T A G C T A T G A G C A T C 400  
RLUCVER1.SEQ T G G G G A G C C T G C C T G G C C C T T C A C T A C T C C T A C G A G C A C C 400  
RLUCVER2.SEQ T G G G G G G C T T G T C T G G C C C T T T C A C T A C T C C T A C G A G C A C C 400  
RLUCFINL.SEQ T G G G G G G C T T G T C T G G C C C T T T C A C T A C T C C T A C G A G C A C C 400

RELLUC.SEQ A A G A T A A G A T C A A A G C A A T A G T T C A C G C T G A A A G T G T A G T 440  
RLUCVER1.SEQ A G A C A A G A T C A A G G C C A T C G T G C A C G C C G A G A G C G T G G T 440  
RLUCVER2.SEQ A A G A C A A G A T C A A G G C C A T C G T C C A T G C T G A G A G T G T C G T 440  
RLUCFINL.SEQ A A G A C A A G A T C A A G G C C A T C G T C C A T G C T G A G A G T G T C G T 440

Figure 7 (Cont.)

RELLUC.SEQ A G A T G T G A T T G A A T C A T G G G A T G A A T G G C C T G A T A T T G A A 480  
RLUCVER1.SEQ G G A C G T G A T C G A G T C C T G G G A C G A G T G G C C T G A C A T C G A G 480  
RLUCVER2.SEQ G G A C G T G A T C G A G T C C T G G G A C G A G T G G C C T G A C A T C G A G 480  
RLUCFINL.SEQ G G A C G T G A T C G A G T C C T G G G A C G A G T G G C C T G A C A T C G A G 480

RELLUC.SEQ G A A G A T A T T G C G T T G A T C A A A T C T G A A G A A G G A G A A A A A A 520  
RLUCVER1.SEQ G A G A C A T C G C C C T G A T C A A G A G C G A G G A G G C G A G A A G A 520  
RLUCVER2.SEQ G A G A T A T C G C C C T G A T C A A G A G C G A A G A G G C G A G A A A A 520  
RLUCFINL.SEQ G A G A T A T C G C C C T G A T C A A G A G C G A A G A G G C G A G A A A A 520

RELLUC.SEQ T G G T T T T G G A G A A T A A C T T C T T C G T G G A A A C C A T G T T G C C 560  
RLUCVER1.SEQ T G G T G C T G G A G A A C A A C T T C T T C G T G G A G A C C A T G C T G C C 560  
RLUCVER2.SEQ T G G T G C T T G A G A A T A A C T T C T T C G T C G A G A C C A T G C T C C C 560  
RLUCFINL.SEQ T G G T G C T T G A G A A T A A C T T C T T C G T C G A G A C C A T G C T C C C 560

RELLUC.SEQ A T C A A A A A T C A T G A G A A A G T T A G A A C C A G A A G A A T T T G C A 600  
RLUCVER1.SEQ C A G C A A G A T C A T G C G C A A G C T G G A G C C T G A G G A G T T C G C C 600  
RLUCVER2.SEQ A A G C A A G A T C A T G C G G A A A C T G G A G C C T G A G G A G T T C G C T 600  
RLUCFINL.SEQ A A G C A A G A T C A T G C G G A A A C T G G A G C C T G A G G A G T T C G C T 600

RELLUC.SEQ G C A T A T C T T G A A C C A T T C A A A G A G A A A G G T G A A G T T C G T C 640  
RLUCVER1.SEQ G C C T A C C T G G A G C C C T T C A A G G A G A A G G G C G A G G T G C G C C 640  
RLUCVER2.SEQ G C C T A C C T G G A G C C C T T C A A G G A G A A G G G C G A G G T T A G A C 640  
RLUCFINL.SEQ G C C T A C C T G G A G C C A T T C A A G G A G A A G G G C G A G G T T A G A C 640

RELLUC.SEQ G T C C A A C A T T A T C A T G G C C T C G T G A A A T C C C G T T A G T A A A 680  
RLUCVER1.SEQ G C C T A C C C T G T C C T G G C C C G C G A G A T C C C T C T G G T G A A 680  
RLUCVER2.SEQ G C C T A C C C T C T C C T G G C C T C G C G A G A T C C C T C T C G T T A A 680  
RLUCFINL.SEQ G C C T A C C C T C T C C T G G C C T C G C G A G A T C C C T C T C G T T A A 680

RELLUC.SEQ A G G T G G T A A A C C T G A C G T T G T A C A A A T T G T T A G G A A T T A T 720  
RLUCVER1.SEQ G G G C G G C A A G C C C G A C G T G G T G C A G A T C G T G C G C A A C T A C 720  
RLUCVER2.SEQ G G G A G G C A A G C C C G A C G T C G T C C A G A T T G T C C G C A A C T A C 720  
RLUCFINL.SEQ G G G A G G C A A G C C C G A C G T C G T C C A G A T T G T C C G C A A C T A C 720

RELLUC.SEQ A A T G C T T A T C T A C G T G C A A G T G A T G A T T T A C C A A A A A T G T 760  
RLUCVER1.SEQ A A C G C C T A C C T G C G C G C C A G C G A C G A C C T G C C T A A G A T G T 760  
RLUCVER2.SEQ A A C G C C T A C C T T C G G G C C A G C G A C G A T C T G C C T A A G A T G T 760  
RLUCFINL.SEQ A A C G C C T A C C T T C G G G C C A G C G A C G A T C T G C C T A A G A T G T 760

RELLUC.SEQ T T A T T G A A T C G G A T C C A G G A T T C T T T T C C A A T G C T A T T G T 800  
RLUCVER1.SEQ T C A T C G A G T C C G A C C C T G G C T T C T T C T C C A A C G C C A T C G T 800  
RLUCVER2.SEQ T C A T C G A G T C C G A C C C T G G G T T C T T T T C C A A C G C T A T T G T 800  
RLUCFINL.SEQ T C A T C G A G T C C G A C C C T G G G T T C T T T T C C A A C G C T A T T G T 800

RELLUC.SEQ T G A A G G C G C C A A G A A G T T T C C T A A T A C T G A A T T T G T C A A A 840  
RLUCVER1.SEQ C G A G G G A G C C A A G A A G T T C C C C A A C A C C G A G T T C G T G A A G 840  
RLUCVER2.SEQ C G A G G G A G C T A A G A A G T T C C C T A A C A C C G A G T T C G T G A A G 840  
RLUCFINL.SEQ C G A G G G A G C T A A G A A G T T C C C T A A C A C C G A G T T C G T G A A G 840

RELLUC.SEQ G T A A A A G G T C T T C A T T T T T C G C A A G A A G A T G C A C C T G A T G 880  
RLUCVER1.SEQ G T G A A G G G C C T G C A C T T C T C C C A G G A G G A C G C C C C T G A C G 880  
RLUCVER2.SEQ G T G A A G G G C C T C C A C T T C A G C C A G G A G G A C G C T C C A G A T G 880  
RLUCFINL.SEQ G T G A A G G G C C T C C A C T T C A G C C A G G A G G A C G C T C C A G A T G 880

## Figure 7 (Cont.)

|              |   |     |
|--------------|---|-----|
| RELUC.SEQ    | A A A T G G G A A A A T A T A T C A A A T C G T T C G T T G A G C G A G T T C T   | 920 |
| RLUCVER1.SEQ | A <u>G</u> A T G G G <u>C</u> A A <u>G</u> T A <u>C</u> A T C A A <u>G A G C</u> T T C G T <u>G</u> G A G C G <u>C</u> G T <u>G</u> C T | 920 |
| RLUCVER2.SEQ | A A A T G G G T A A <u>G</u> T A <u>C</u> A T C A A <u>G A G C</u> T T C G T <u>G</u> G A G C G <u>C</u> G T <u>G</u> C T               | 920 |
| RLUCFINL.SEQ | A A A T G G G T A A <u>G</u> T A <u>C</u> A T C A A <u>G A G C</u> T T C G T <u>G</u> G A G C G <u>C</u> G T <u>G</u> C T               | 920 |
|              |   |     |
| RELUC.SEQ    | C A A A A A T G A A C A A   | 933 |
| RLUCVER1.SEQ | <u>G</u> A A <u>G</u> A A <u>C</u> G A <u>G</u> C A <u>G</u>  | 933 |
| RLUCVER2.SEQ | <u>G</u> A A <u>G</u> A A <u>C</u> G A <u>G</u> C A <u>G</u>  | 933 |
| RLUCFINL.SEQ | <u>G</u> A A <u>G</u> A A <u>C</u> G A <u>G</u> C A <u>G</u>  | 933 |

## Figure 8

RELLUC.SEQ M T S K V Y D P E Q R K R M I T G P Q W W A R C K Q M N V L D S F I N Y Y D S E 118  
RLUCVER1.SEQ M A S K V Y D P E Q R K R M I T G P Q W W A R C K Q M N V L D S F I N Y Y D S E 118  
RLUCVER2.SEQ M A S K V Y D P E Q R K R M I T G P Q W W A R C K Q M N V L D S F I N Y Y D S E 118  
RLUCFINL.SEQ M A S K V Y D P E Q R K R M I T G P Q W W A R C K Q M N V L D S F I N Y Y D S E 118

RELLUC.SEQ K H A E N A V I F L H G N A A S S Y L W R H V V P H I E P V A R C I I P D L I G 238  
RLUCVER1.SEQ K H A E N A V I F L H G N A A S S Y L W R H V V P H I E P V A R C I I P D L I G 238  
RLUCVER2.SEQ K H A E N A V I F L H G N A A S S Y L W R H V V P H I E P V A R C I I P D L I G 238  
RLUCFINL.SEQ K H A E N A V I F L H G N A A S S Y L W R H V V P H I E P V A R C I I P D L I G 238

RELLUC.SEQ M G K S G K S G N G S Y R L L D H Y K Y L T A W F E L L N L P K K I I F V G H D 358  
RLUCVER1.SEQ M G K S G K S G N G S Y R L L D H Y K Y L T A W F E L L N L P K K I I F V G H D 358  
RLUCVER2.SEQ M G K S G K S G N G S Y R L L D H Y K Y L T A W F E L L N L P K K I I F V G H D 358  
RLUCFINL.SEQ M G K S G K S G N G S Y R L L D H Y K Y L T A W F E L L N L P K K I I F V G H D 358

RELLUC.SEQ W G A C L A F H Y S Y E H Q D K I K A I V H A E S V V D V I E S W D E W P D I E 478  
RLUCVER1.SEQ W G A C L A F H Y S Y E H Q D K I K A I V H A E S V V D V I E S W D E W P D I E 478  
RLUCVER2.SEQ W G A C L A F H Y S Y E H Q D K I K A I V H A E S V V D V I E S W D E W P D I E 478  
RLUCFINL.SEQ W G A C L A F H Y S Y E H Q D K I K A I V H A E S V V D V I E S W D E W P D I E 478

RELLUC.SEQ E D I A L I K S E E G E K M V L E N N F F V E T M L P S K I M R K L E P E E F A 598  
RLUCVER1.SEQ E D I A L I K S E E G E K M V L E N N F F V E T M L P S K I M R K L E P E E F A 598  
RLUCVER2.SEQ E D I A L I K S E E G E K M V L E N N F F V E T M L P S K I M R K L E P E E F A 598  
RLUCFINL.SEQ E D I A L I K S E E G E K M V L E N N F F V E T M L P S K I M R K L E P E E F A 598

RELLUC.SEQ A Y L E P F K E K G E V R R P T L S W P R E I P L V K G G K P D V V Q I V R N Y 718  
RLUCVER1.SEQ A Y L E P F K E K G E V R R P T L S W P R E I P L V K G G K P D V V Q I V R N Y 718  
RLUCVER2.SEQ A Y L E P F K E K G E V R R P T L S W P R E I P L V K G G K P D V V Q I V R N Y 718  
RLUCFINL.SEQ A Y L E P F K E K G E V R R P T L S W P R E I P L V K G G K P D V V Q I V R N Y 718

RELLUC.SEQ N A Y L R A S D D L P K M F I E S D P G F F S N A I V E G A K K F P N T E F V K 838  
RLUCVER1.SEQ N A Y L R A S D D L P K M F I E S D P G F F S N A I V E G A K K F P N T E F V K 838  
RLUCVER2.SEQ N A Y L R A S D D L P K M F I E S D P G F F S N A I V E G A K K F P N T E F V K 838  
RLUCFINL.SEQ N A Y L R A S D D L P K M F I E S D P G F F S N A I V E G A K K F P N T E F V K 838

RELLUC.SEQ V K G L H F S Q E D A P D E M G K Y I K S F V E R V L K N E Q 931  
RLUCVER1.SEQ V K G L H F S Q E D A P D E M G K Y I K S F V E R V L K N E Q 931  
RLUCVER2.SEQ V K G L H F S Q E D A P D E M G K Y I K S F V E R V L K N E Q 931  
RLUCFINL.SEQ V K G L H F S Q E D A P D E M G K Y I K S F V E R V L K N E Q 931

## Figure 9A

Codon usage in RELLUC

(*Renilla reniformis*; Genbank ACCESSION:M63501; Medline:91239583)

|     |     |    |     |     |    |     |     |    |     |     |    |
|-----|-----|----|-----|-----|----|-----|-----|----|-----|-----|----|
| TTT | Phe | 11 | TCT | Ser | 5  | TAT | Tyr | 12 | TGT | Cys | 3  |
| TTC | Phe | 5  | TCC | Ser | 1  | TAC | Tyr | 1  | TGC | Cys | 0  |
| TTA | Leu | 8  | TCA | Ser | 6  | TAA | *** | 0  | TGA | *** | 0  |
| TTG | Leu | 4  | TCG | Ser | 4  | TAG | *** | 0  | TGG | Trp | 8  |
| CTT | Leu | 8  | CCT | Pro | 5  | CAT | His | 9  | CGT | Arg | 4  |
| CTC | Leu | 1  | CCC | Pro | 0  | CAC | His | 1  | CGC | Arg | 0  |
| CTA | Leu | 1  | CCA | Pro | 11 | CAA | Gln | 6  | CGA | Arg | 2  |
| CTG | Leu | 0  | CCG | Pro | 2  | CAG | Gln | 1  | CGG | Arg | 2  |
| ATT | Ile | 12 | ACT | Thr | 4  | AAT | Asn | 11 | AGT | Ser | 2  |
| ATC | Ile | 6  | ACC | Thr | 1  | AAC | Asn | 2  | AGC | Ser | 1  |
| ATA | Ile | 3  | ACA | Thr | 1  | AAA | Lys | 21 | AGA | Arg | 2  |
| ATG | Met | 9  | ACG | Thr | 0  | AAG | Lys | 6  | AGG | Arg | 3  |
| GTT | Val | 12 | GCT | Ala | 5  | GAT | Asp | 16 | GGT | Gly | 10 |
| GTC | Val | 2  | GCC | Ala | 3  | GAC | Asp | 1  | GGC | Gly | 4  |
| GTA | Val | 6  | GCA | Ala | 8  | GAA | Glu | 25 | GGA | Gly | 3  |
| GTG | Val | 3  | GCG | Ala | 3  | GAG | Glu | 5  | GGG | Gly | 0  |

## Figure 9B

## Codon Usage in Rluc-final

|     |     |    |     |     |    |     |     |    |     |     |   |
|-----|-----|----|-----|-----|----|-----|-----|----|-----|-----|---|
| TTT | Phe | 4  | TCT | Ser | 0  | TAT | Tyr | 2  | TGT | Cys | 1 |
| TTC | Phe | 12 | TCC | Ser | 10 | TAC | Tyr | 11 | TGC | Cys | 2 |
| TTA | Leu | 0  | TCA | Ser | 1  | TAA | *** | 0  | TGA | *** | 0 |
| TTG | Leu | 0  | TCG | Ser | 0  | TAG | *** | 0  | TGG | Trp | 8 |
| CTT | Leu | 3  | CCT | Pro | 11 | CAT | His | 2  | CGT | Arg | 0 |
| CTC | Leu | 6  | CCC | Pro | 3  | CAC | His | 8  | CGC | Arg | 7 |
| CTA | Leu | 0  | CCA | Pro | 4  | CAA | Gln | 3  | CGA | Arg | 0 |
| CTG | Leu | 13 | CCG | Pro | 0  | CAG | Gln | 4  | CGG | Arg | 3 |
| ATT | Ile | 3  | ACT | Thr | 1  | AAT | Asn | 2  | AGT | Ser | 1 |
| ATC | Ile | 18 | ACC | Thr | 4  | AAC | Asn | 11 | AGC | Ser | 7 |
| ATA | Ile | 0  | ACA | Thr | 0  | AAA | Lys | 4  | AGA | Arg | 2 |
| ATG | Met | 9  | ACG | Thr | 0  | AAG | Lys | 23 | AGG | Arg | 1 |
| GTT | Val | 2  | GCT | Ala | 11 | GAT | Asp | 6  | GGT | Gly | 3 |
| GTC | Val | 8  | GCC | Ala | 9  | GAC | Asp | 11 | GGC | Gly | 7 |
| GTA | Val | 0  | GCA | Ala | 0  | GAA | Glu | 2  | GGA | Gly | 3 |
| GTG | Val | 13 | GCG | Ala | 0  | GAG | Glu | 28 | GGG | Gly | 4 |

## Figure 10

Oligonucleotides for the assembly of synthetic *Renilla* luciferase gene

### Sense Strand

| Oligo name      | Oligo sequence from 5' to 3'                      |                 |
|-----------------|---|-----------------|
| RLS1 (1-40)     | AACCATGGCTTCCAAGGTGTACGACCCCGAGCAACGCAAA          | (SEQ ID NO:246) |
| RLS2 (41-80)    | CGCATGATCACTGGGCCCTCAGTGGTGGGCTCGCTGCAAGC         | (SEQ ID NO:247) |
| RLS3 (81-120)   | AAATGAACGTGCTGGACTCCTTCATCAACTACTATGATTC          | (SEQ ID NO:248) |
| RLS4 (121-170)  | CGAGAAGCACGCCGAGAACGCCGTGATTTTCTGCATGGTAACGCTGCCT | (SEQ ID NO:249) |
| RLS5 (171-210)  | CCAGCTACCTGTGGAGGCACGTCGTGCCTCACATCGAGCC          | (SEQ ID NO:250) |
| RLS6 (211-250)  | CGTGGCTAGATGCATCATCCCTGATCTGATCGGAATGGGT          | (SEQ ID NO:251) |
| RLS7 (251-290)  | AAGTCCGGCAAGAGCGGGAATGGCTCATATCGCCTCCTGG          | (SEQ ID NO:252) |
| RLS8 (291-330)  | ATCACTACAAGTACCTCACCGCTTGGTTCGAGCTGCTGAA          | (SEQ ID NO:253) |
| RLS9 (331-370)  | CCTTCCAAAGAAAATCATCTTTGTGGGCCACGACTGGGGG          | (SEQ ID NO:254) |
| RLS10 (371-410) | GCTTGTCTGGCCTTTCCTACTCTCTACGAGCACCAAGACA          | (SEQ ID NO:255) |
| RLS11 (411-450) | AGATCAAGGCCATCGTCCATGCTGAGAGTGTCTGTTGACGT         | (SEQ ID NO:256) |
| RLS12 (451-495) | GATCGAGTCCTGGGACGAGTGGCCTGACATCGAGGAGGATATCGC     | (SEQ ID NO:257) |
| RLS13 (496-535) | CCTGATCAAGAGCGAAGAGGGCGAGAAAATGGTGTCTGAG          | (SEQ ID NO:258) |
| RLS14 (536-575) | AATAACTTCTTCGTGAGACCATGCTCCCAAGCAAGATCA           | (SEQ ID NO:259) |
| RLS15 (576-620) | TGCGGAACTGGAGCCTGAGGAGTTCGCTGCCTACCTGGAGCCAT      | (SEQ ID NO:260) |
| RLS16 (621-660) | TCAAGGAGAAGGGCGAGGTTAGACGGCCTACCTCTCTCTG          | (SEQ ID NO:261) |
| RLS17 (661-700) | GCCTCGCGAGATCCCTCTCGTTAAGGGAGGCAAGCCCGAC          | (SEQ ID NO:262) |
| RLS18 (701-740) | GTCGTCCAGATTGTCCGCAACTACAACGCCTACCTTCGGG          | (SEQ ID NO:263) |
| RLS19 (741-780) | CCAGCGACGATCTGCCTAAGATGTTTCATCGAGTCCGACCC         | (SEQ ID NO:264) |
| RLS20 (781-820) | TGGGTTCTTTTCCAACGCTATTGTGCGAGGGAGCTAAGAAG         | (SEQ ID NO:265) |
| RLS21 (821-860) | TTCCCTAACACCGAGTTCGTGAAGGTGAAGGGCCTCCACT          | (SEQ ID NO:266) |
| RLS22 (861-900) | TCAGCCAGGAGGACGCTCCAGATGAAATGGGTAAAGTACAT         | (SEQ ID NO:267) |
| RLS23 (901-949) | CAAGAGCTTCGTGGAGCGCGTCTGAAGAACGAGCAGTAATTCTAGAGC  | (SEQ ID NO:268) |

### Anti-sense Strand

| Oligo name       | Oligo Sequence from 5' to 3'                  |                 |
|------------------|---|-----------------|
| RLAS1 (1-29)     | GCTCTAGAATTACTGCTCGTTCTTCAGCA                 | (SEQ ID NO:269) |
| RLAS2 (30-69)    | CGCGTCCACGAAAGCTCTTGATGTACTTACCCATTTCATC      | (SEQ ID NO:270) |
| RLAS3 (70-109)   | TGGAGCGTCCCTCGTGGCTGAAGTGGAGGCCCTTCACCTTC     | (SEQ ID NO:271) |
| RLAS4 (110-149)  | ACGAACTCGGTGTTAGGGAACCTTCTAGCTCCCTCGACAA      | (SEQ ID NO:272) |
| RLAS5 (150-189)  | TAGCGTTGGAAAAGAACCCAGGGTTCGGAATCGATGAACAT     | (SEQ ID NO:273) |
| RLAS6 (190-229)  | CTTAGGCAGATCGTCTGCTGGCCCGAAGGTAGGCGTTGTAG     | (SEQ ID NO:274) |
| RLAS7 (230-269)  | TTGCGGACAATCTGGACGACGTCGGGCTTGCTCCCTTAA       | (SEQ ID NO:275) |
| RLAS8 (270-309)  | CGAGAGGGATCTCGCGAGGCCAGGAGAGGGTAGGCCGTCT      | (SEQ ID NO:276) |
| RLAS9 (310-349)  | AACCTCGCCCTTCTCCTTGAATGGCTCCAGGTAGGCAGCG      | (SEQ ID NO:277) |
| RLAS10 (350-394) | AACTCCTCAGGCTCCAGTTTCCGCATGATCTTGCTTGGGAGCATG | (SEQ ID NO:278) |
| RLAS11 (395-434) | GTCTCGACGAAGAAGTTATTCTCAAGCACCATTTTCTCGC      | (SEQ ID NO:279) |
| RLAS12 (435-474) | CCTCTTCGCTCTTGATCAGGGCGATATCCTCCTCGATGTC      | (SEQ ID NO:280) |
| RLAS13 (475-517) | AGGCCACTCGTCCAGGACTCGATCACGTCCACGACACTCTCA    | (SEQ ID NO:281) |
| RLAS14 (518-559) | GCATGGACGATGGCCTTGATCTTGTCTTGGTGCTCGTAGGAG    | (SEQ ID NO:282) |
| RLAS15 (560-599) | TAGTGAAAGGCCAGACAAGCCCCCAGTCGTGGCCACAA        | (SEQ ID NO:283) |
| RLAS16 (600-639) | AGATGATTTTCTTTGGAAGGTTTCAGCAGCTCGAACCAAGC     | (SEQ ID NO:284) |
| RLAS17 (640-679) | GGTGAGGTACTTGTAGTGATCCAGGAGGCGATATGAGCCA      | (SEQ ID NO:285) |
| RLAS18 (680-719) | TTCCCGCTCTTGCCCGACTTACCCATTCCGATCAGATCAG      | (SEQ ID NO:286) |
| RLAS19 (720-764) | GGATGATGCATCTAGCCACGGGCTCGATGTGAGGCACGACGTGCC | (SEQ ID NO:287) |
| RLAS20 (765-804) | TCCACAGGTAGCTGGAGGCGAGCGTTACCATGCAGAAAAAT     | (SEQ ID NO:288) |
| RLAS21 (805-849) | CACGGCGTTCTCGGCGTGCTTCTCGGAATCATAGTAGTTGATGAA | (SEQ ID NO:289) |
| RLAS22 (850-889) | GGAGTCCAGCAGGTTTCAATTTGCTTGCAGCGAGCCCACCAC    | (SEQ ID NO:290) |
| RLAS23 (890-929) | TGAGGCCAGTGATCATGCGTTTTCGTTGCTCGGGGTCGT       | (SEQ ID NO:291) |
| RLAS24 (930-949) | ACACCTTGGAAGCCATGGTT                          | (SEQ ID NO:292) |



Figure 11

GRVER51.SEQ A T G A T G A A A C G C G A A A A G A A C G T G A T C T A C G G C C A G A A C 40  
LUCPPPLYG.SEQ A T G A T G A A G A G A G A G A A A A A T G T T A T A T A T G G A C C C G A A C 40  
RD1561H9.SEQ A T G A T A A A G C G T G A G A A A A A T G T C A T C T A T G G C C C T G A G C 40

GRVER51.SEQ C A C T G C A T C C A C T G G A A G A C C T C A C C G C T G G T G A G A T G C T 80  
LUCPPPLYG.SEQ C C C T A C A C C C C T T G G A A G A C T T A A C A G C A G G A G A A A T G C T 80  
RD1561H9.SEQ C T C T C A T C C T T T G G A G G A T T T G A C T G C C G G C G A A A T G C T 80

GRVER51.SEQ C T T C C G A G C A C T G C G T A A A C A T A G T C A C C T C C C T C A A G C A 120  
LUCPPPLYG.SEQ C T T C A G G G C C C T T C G A A A C A T T C T C A T T T A C C G C A G G C T 120  
RD1561H9.SEQ G T T T C G T G C T C T C C G C A A G C A C T C T C A T T T G C C T C A A G C C 120

GRVER51.SEQ C T C G T G G A C G T C G T G G G A G A C G A G A G C C T C T C C T A C A A A G 160  
LUCPPPLYG.SEQ T T A G T A G A T G T G T T T G G T G A C G A A T C G C T T T C C T A T A A A G 160  
RD1561H9.SEQ T T G G T C G A T G T G T C G G C G A T G A A T C T T T G A G C T A C A A G G 160

GRVER51.SEQ A A T T T T T C G A A G C T A C T G T G C T G T T G G C C C A A A G C C T C C A 200  
LUCPPPLYG.SEQ A G T T T T T T G A A G C T A C A T G C C T C C T A G C G C A A A G T C T C C A 200  
RD1561H9.SEQ A G T T T T T T G A G G C A A C C G T C T T G C T G G C T C A G T C C C T C C A 200

GRVER51.SEQ T A A T T G T G G G T A C A A A A T G A A C G A T G T G G T G A G C A T T T G T 240  
LUCPPPLYG.SEQ C A A T T G T G G A T A C A A G A T G A A T G A T G T A G T G T C G A T C T G C 240  
RD1561H9.SEQ C A A T T G T G G C T A C A A G A T G A A C G A C G T C G T T A G T A T C T G T 240

GRVER51.SEQ G C T G A G A A T A A C A C T C G C T T C T T T A T T C C T G T A A T C G C T G 280  
LUCPPPLYG.SEQ G C C G A G A A T A A T A A A A G A T T T T T T A T T C C C A T T A T T G C A G 280  
RD1561H9.SEQ G C T G A A A A C A A T A C C C G T T T C T T C A T T C C A G T C A T C G C C G 280

GRVER51.SEQ C T T G G T A C A T C G G C A T G A T T G T C G C C C C T G T G A A T G A A T C 320  
LUCPPPLYG.SEQ C T T G G T A T A T T G G T A T G A T T G T A G C A C C T G T T A A T G A A A G 320  
RD1561H9.SEQ C A T G G T A T A T C G G T A T G A T C G T G G C T C C A G T C A A C G A G A G 320

GRVER51.SEQ T T A C A T C C C A G A T G A G C T G T G T A A G G T T A T G G G T A T T A G C 360  
LUCPPPLYG.SEQ T T A C A T C C C A G A T G A A C T C T G T A A G G T C A T G G G T A T A T C G 360  
RD1561H9.SEQ C T A C A T T C C C G A C G A A C T G T G T A A A G T C A T G G G T A T C T C T 360

GRVER51.SEQ A A A C C T C A A A T C G T C T T T A C T A C C A A A A A C A T C T T G A A T A 400  
LUCPPPLYG.SEQ A A A C C A C A A A T A G T T T T T T G T A C A A A G A A C A T T T T A A A T A 400  
RD1561H9.SEQ A A G C C A C A G A T T G T C T T C A C C A C T A A G A A T A T T C T G A A C A 400

GRVER51.SEQ A G G T C T T G G A A G T C A G T C T C T G T A C T A A C T T C A T C A A A C G 440  
LUCPPPLYG.SEQ A G G T A T T G G A G G T A C A G A G C A G A A C T A A T T T C A T A A A A A G 440  
RD1561H9.SEQ A A G T C C T G G A A G T C C A A A G C C G C A C C A A C T T T A T T A A G C G 440

GRVER51.SEQ C A T C A T T A T T C T G G A T A C C G T C G A A A A C A T C C A C G G C T G T 480  
LUCPPPLYG.SEQ G A T C A T C A T A C T T G A T A C T G T A G A A A A C A T A C A C G G T T G T 480  
RD1561H9.SEQ T A T C A T C A T C T T G G A C A C T G T G G A G A A T A T T C A C G G T T G C 480

GRVER51.SEQ G A G A G C C T C C C T A A C T T C A T C T C T C G T T A C A G C G A T G G T A 520  
LUCPPPLYG.SEQ G A A A G T C T T C C C A A T T T T A T T T C T C G T T A T T C G G A T G G A A 520  
RD1561H9.SEQ G A A T C T T T G C C T A A T T T C A T C T C T C G C T A T T C A G A C G G C A 520

GRVER51.SEQ A T A T C G C T A A T T T C A A G C C C T T G C A T T T T G A T C C A G T C G A 560  
LUCPPPLYG.SEQ A T A T T G C C A A C T T C A A A C C T T A C A T T A C G A T C C T G T T G A 560  
RD1561H9.SEQ A C A T C G C A A A C T T T A A A C C A C T C C A C T T C G A C C C T G T G G A 560

Figure 11 (Cont.)

GRVER51.SEQ G C A A G T G G C [C] G C T A T [T] T T [G] T G [C] T C [C] T C [C] G G C A C [C] A C T G G [T] 600  
LUCPLYG.SEQ G C A A G T G G C A G C T A T C T T A T G T T C G T C A G G C A C T A C T G G A 600  
RD1561H9.SEQ [A] C A A G T [T] G C A G C [C] A T [T] C [T] G T G T [A] G C A G C [C] G G [T] A C T A C T G G A 600

GRVER51.SEQ T T [G] C C [T] A A A G G T G T [C] A T G C A [G] A C T C A C C A [G] A A T A T [C] T G T G 640  
LUCPLYG.SEQ T T A C C G A A A G G T G T A A T G C A A A C T C A C C A A A A T A T T T G T G 640  
RD1561H9.SEQ [C] T [C] C C [A] A A [G] G G [A] G T [C] A T G C A [G] A C [C] C A [T] C A A A A [C] A T T T G [C] G 640

GRVER51.SEQ T [G] C G [T] T [T] G A T [C] C A [C] G C T [C] T [C] G A C C C [T] C [G] T [G] T G G [T] A C [T] C A 680  
LUCPLYG.SEQ T C C G A C T T A T A C A T G C T T T A G A C C C C A G G G C A G G A A C G C A 680  
RD1561H9.SEQ T [G] C G [T] C T [G] A T [C] C A T G C T [C] T [C] G A [T] C C [A] C [G] C T A C [G] G [C] A C [T] C A 680

GRVER51.SEQ A [T] T [G] A T [C] C C T G G [C] G T G A C [T] G T [G] C T [G] G T [G] T A T C T G C C T T T [C] 720  
LUCPLYG.SEQ A C T T A T T C C T G G T G T G A C A G T C T T A G T A T A T C T G C C T T T T 720  
RD1561H9.SEQ [G] C T [G] A T T C C T G G T G T [C] A C [C] G T C T T [G] G T [C] T A [C] T T G C C T T T [C] 720

GRVER51.SEQ T T [T] C A [C] G C [C] T T T G G [T] T T C T C T A T [T] A [C] C [C] T G G G [C] T A [T] T T C A 760  
LUCPLYG.SEQ T T C C A T G C T T T T G G G T T C T C T A T A A A C T T G G G A T A C T T C A 760  
RD1561H9.SEQ T T C C A T G C T T T [C] G G [C] T T [T] C A T A T [T] A [C] T T T G G G [T] T A C T T [T] A 760

GRVER51.SEQ T G G T [C] G G [C] T [T] G C G T G T [C] A T C A T G T T [T] C [G] T [C] G [C] T T [C] G A [C] C A 800  
LUCPLYG.SEQ T G G T G G G T C T T C G T G T T A T C A T G T T A A G A C G A T T T G A T C A 800  
RD1561H9.SEQ T G G T [C] G G T C T [C] C G [C] G T [G] A T [T] A T G T T [C] C [G] [C] C G [T] T T T G A T C A 800

GRVER51.SEQ A G A A G C [C] T T [C] T [T] G A A [G] G C T A T T C A [A] G A [C] T A [C] G A [G] G T [G] C G [T] 840  
LUCPLYG.SEQ A G A A G C A T T T C T A A A A G C T A T T C A G G A T T A T G A A G T T C G A 840  
RD1561H9.SEQ [G] G A [G] G C [T] T T [C] T [T] G A A A G C [C] A T [C] C A [A] G A T T A T G A A G T [C] C G [C] 840

GRVER51.SEQ [T] C [C] G T [G] A T [C] A A C G T [C] C C [T] T C A [G] T [C] A T [T] T G T T C [C] T [G] A G C A 880  
LUCPLYG.SEQ A G T G T A A T T A A C G T T C C A G C A A T A A T A T T G T T C T T A T C G A 880  
RD1561H9.SEQ A G T G T [C] A T [C] A A C G T [G] C C [T] A G C G T [G] A T [C] C T G T T [T] T T [G] T C [T] A 880

GRVER51.SEQ A A [T] C [T] C C T T T G G T T G A C A A [G] T A [T] G A T [C] T [G] A G C A G [C] T T [G] C G 920  
LUCPLYG.SEQ A A A G T C C T T T G G T T G A C A A A T A C G A T T T A T C A A G T T T A A G 920  
RD1561H9.SEQ A [G] A G [C] C C [A] C T [C] G T [G] G A C A A [G] T A C G A [C] T T [G] T C [T] T C A C T [G] C G 920

GRVER51.SEQ [T] G A [G] C [T] G T G [C] T G [T] G G [C] G C [T] G C [T] C C [T] T T [G] G C [C] A A A G A A G T [G] 960  
LUCPLYG.SEQ G G A A T T G T G T T G C G G T G C G G C A C C A T T A G C A A A A G A A G T T 960  
RD1561H9.SEQ [T] G A A T T G T G T T G C G G T G C [C] G C [T] C C A [C] T [G] G C [T] A A [G] G A [G] G T [C] 960

GRVER51.SEQ G C [C] G A G G T [C] G C [T] G [C] T A A [G] C G [T] C T [G] A A C [C] T [C] C C [T] G G [T] A T [C] C 1000  
LUCPLYG.SEQ G C T G A G G T T G C A G T A A A A C G A T T A A A C T T G C C A G G A A T T C 1000  
RD1561H9.SEQ G C T G A [A] G T [G] G C [C] G [C] C A A A C G [C] T T [G] A A T [C] T [T] C C A G G [G] A T T C 1000

GRVER51.SEQ G C T G [C] G G [T] T T T G G T T T G A C [T] G A [G] A G C A C T T C [T] G C T A A [C] A T 1040  
LUCPLYG.SEQ G C T G T G G A T T T G G T T T G A C A G A A T C T A C T T C A G C T A A T A T 1040  
RD1561H9.SEQ G [T] T G T G G [C] T T [C] G G [C] C T [C] A C [C] G A A T C T A C [C] A G T G C [G] A [T] T A T 1040

GRVER51.SEQ [C] C A [T] A G [C] T T [G] C G [A] G A [C] G A [G] T T T A A [G] T C [T] G G [T] A G C [C] T [G] G G [T] 1080  
LUCPLYG.SEQ A C A C A G T C T T G G G G A T G A A T T T A A A T C A G G A T C A C T T G G A 1080  
RD1561H9.SEQ [C] C A [G] A [C] T C T [C] G G G G A T G A [G] T T T A A [G] A G C [G] G [C] T C [T] T T [G] G G [C] 1080

GRVER51.SEQ [C] G [C] G T [G] A C T C C T [C] T T A T G G C [T] G C [A] A A [G] A T [C] G C [C] G A [C] C [G] T G 1120  
LUCPLYG.SEQ A G A G T T A C T C C T T T A A T G G C A G C T A A A A T A G C A G A T A G G G 1120  
RD1561H9.SEQ [C] G [T] G T [C] A C T C C [A] C T [C] A T G G C [T] G C T A A [G] A T [C] G C [T] G A T [C] G [C] G 1120

Figure 11 (Cont.)

GRVER51.SEQ A G A C G G C A A A G C A T G G G C C C A A A T C A A G T C G G T G A A T T 1160  
LUCPPLYG.SEQ A A A C T G G T A A A G C A T T G G G A C C A A A T C A A G T T G G T G A A T T 1160  
RD1561H9.SEQ A A A C T G G T A A G G C T T T G G G C C C G A A C C A A G T G G G C G A G C T 1160

GRVER51.SEQ G T G T A T T A A G G G C C C T A T G G T C T C T A A A G G C T A C G T G A A C 1200  
LUCPPLYG.SEQ A T G C G T T A A A G G T C C C A T G G T A T C G A A A G G T T A C G T G A A C 1200  
RD1561H9.SEQ G T G T A T C A A A G G G C C C T A T G G T G A G C A A G G G T T A T G T C A A T 1200

GRVER51.SEQ A A T G T G G A G G C C A C T A A A G A A G C C A T T G A T G A T G A T G G C T 1240  
LUCPPLYG.SEQ A A T G T A G A A G C T A C C A A A G A A G C T A T T G A T G A T G A T G G T T 1240  
RD1561H9.SEQ A A C G T T G A A G C T A C C A A G G A G G C C A T C G A C G A C G A C G G C T 1240

GRVER51.SEQ G G C T C C A T A G C G G C G A C T T C G G T T A C T A T G A T G A G G A C G A 1280  
LUCPPLYG.SEQ G G C T T C A C T C T G G A G A C T T T G G A T A C T A T G A T G A G G A T G A 1280  
RD1561H9.SEQ G G T T G C A T T C T G G T G A T T T T G G A T A T T A C G A C G A A G A T G A 1280

GRVER51.SEQ A C A C T T C T A T G T G G T C G A T C G C T A C A A A G A A T T G A T T A A G 1320  
LUCPPLYG.SEQ G C A T T T C T A T G T G G T G G A C C G T T A C A A G G A A T T G A T T A A A 1320  
RD1561H9.SEQ G C A T T T T A C G T C G T G G A T C G T T A C A A G G A G C T G A T C A A A 1320

GRVER51.SEQ T A C A A A G G C T C T C A A G T C G C A C C A G C C G A A C T G G A A G A A A 1360  
LUCPPLYG.SEQ T A T A A G G G C T C T C A G G T A G C A C C T G C A G A A C T A G A A G A G A 1360  
RD1561H9.SEQ T A C A A G G G T A G C C A G G T T G C T C C A G C T G A G T T G G A G G A G A 1360

GRVER51.SEQ T T T T G C T G A A G A A C C C T T G T A T C C G C G A C G T G G C C G T C G T 1400  
LUCPPLYG.SEQ T T T T A T T G A A A A A T C C A T G T A T C A G A G A T G T T G C T G T G G T 1400  
RD1561H9.SEQ T T C T G T T G A A A A A T C C A T G C A T T C G C G A T G T C G C T G T G G T 1400

GRVER51.SEQ G G G T A T C C C A G A C T T G G A A G C T G G C G A G T T G C C T A G C G C 1440  
LUCPPLYG.SEQ T G G T A T T C C T G A T C T A G A A G C T G G A G A A C T G C C A T C T G C G 1440  
RD1561H9.SEQ C G G C A T T C C T G A T C T G G A G G C C G G C G A A C T G C C T T C T G C T 1440

GRVER51.SEQ T T T G T G G T G A A A C A A C C C G G C A A G G A G A T C A C T G C T A A G G 1480  
LUCPPLYG.SEQ T T T G T G G T T A A A C A G C C C G G A A A G G A G A T T A C A G C T A A A G 1480  
RD1561H9.SEQ T T C G T T G T C A A G C A G C C T G G T A C A G A A A T T A C C G C C A A A G 1480

GRVER51.SEQ A G G T C T A C G A C T A T T T G G C C G A G C G C G T G T C T C A C A C C A A 1520  
LUCPPLYG.SEQ A A G T G T A C G A T T A T C T T G C C G A G A G G G T C T C C C A T A C A A A 1520  
RD1561H9.SEQ A A G T G T A T G A T T A C T G G C T G A A C G T G T G A G C C A T A C T A A 1520

GRVER51.SEQ A T A T C T G C G T G G C G G C G T C C G C T T C G T C G A T T C T A T T C C A 1560  
LUCPPLYG.SEQ G T A T T T G C G T G G A G G G G T T C G A T T C G T T G A T A G C A T A C C A 1560  
RD1561H9.SEQ G T A C T T G C G T G G C G G C G T G C G T T T T G T T G A C T C C A T C C C T 1560

GRVER51.SEQ C G C A A C G T T A C C G G T A A G A T C A C T C G T A A A G A G T T G C T G A 1600  
LUCPPLYG.SEQ A G G A A T G T T A C A G G T A A A A T T A C A A G A A A G G A A C T T C T G A 1600  
RD1561H9.SEQ C G T A A C G T A A C A G G C A A A A T T A C C C G C A A G G A G C T G T T G A 1600

GRVER51.SEQ A G C A A C T C C T C G A A A A A G C T G G C G G C 1626  
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## Figure 12

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 RD1561H9.SEQ M **I** K R E K N V I Y G P E P L H P L E D L T A G E M L F R A L R K H S H L P Q A 118

GRVER51.SEQ L V D V **V** G D E S L S Y K E F F E A T **V** L L A Q S L H N C G Y K M N D V V S I C 238  
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 RD1561H9.SEQ L V D V **V** G D E S L S Y K E F F E A T **V** L L A Q S L H N C G Y K M N D V V S I C 238

GRVER51.SEQ A E N N **T** R F F I P **V** I A A W Y I G M I V A P V N E S Y I P D E L C K V M G I S 358  
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 RD1561H9.SEQ A E N N **T** R F F I P **V** I A A W Y I G M I V A P V N E S Y I P D E L C K V M G I S 358

GRVER51.SEQ K P Q I V F **T** T K N I L N K V L E V Q S R T N F I K R I I I L D T V E N I H G C 478  
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 RD1561H9.SEQ N V E A T K E A I D D D G W L H S G D F G Y Y D E D E H F Y V V D R Y K E L I K 1318

GRVER51.SEQ Y K G S Q V A P A E L E E I L L K N P C I R D V A V V G I P D L E A G E L P S A 1438  
 LUCPPPLYG.SEQ Y K G S Q V A P A E L E E I L L K N P C I R D V A V V G I P D L E A G E L P S A 1438  
 RD1561H9.SEQ Y K G S Q V A P A E L E E I L L K N P C I R D V A V V G I P D L E A G E L P S A 1438

GRVER51.SEQ F V V K Q P G K E I T A K E V Y D Y L A E R V S H T K Y L R G G V R F V D S I P 1558  
 LUCPPPLYG.SEQ F V V K Q P G K E I T A K E V Y D Y L A E R V S H T K Y L R G G V R F V D S I P 1558  
 RD1561H9.SEQ F V V K Q P G **T** E I T A K E V Y D Y L A E R V S H T K Y L R G G V R F V D S I P 1558

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 LUCPPPLYG.SEQ R N V T G K I T R K E L L K Q L L E K S S K L 1627  
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# Renilla luciferase gene in pGL3 series

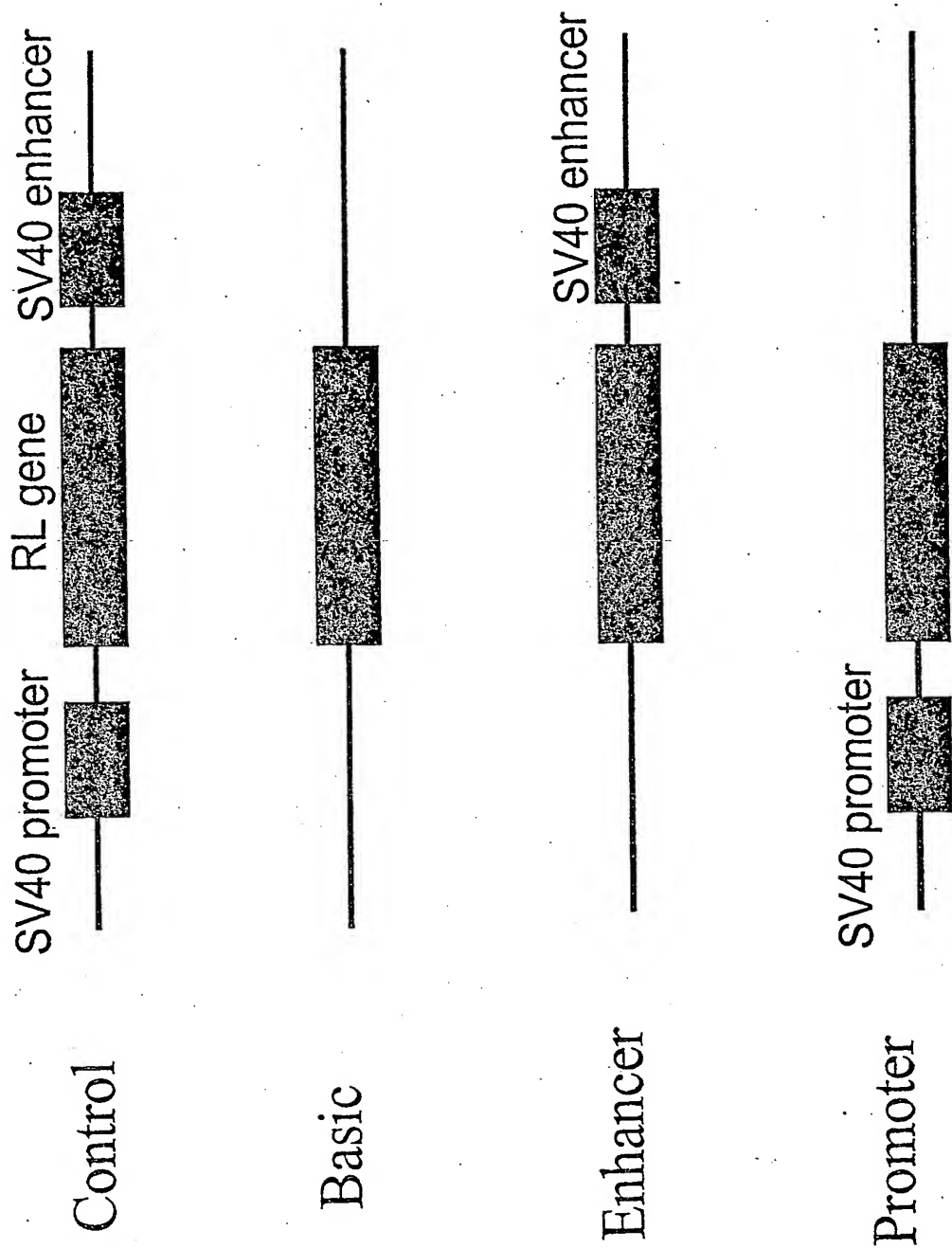
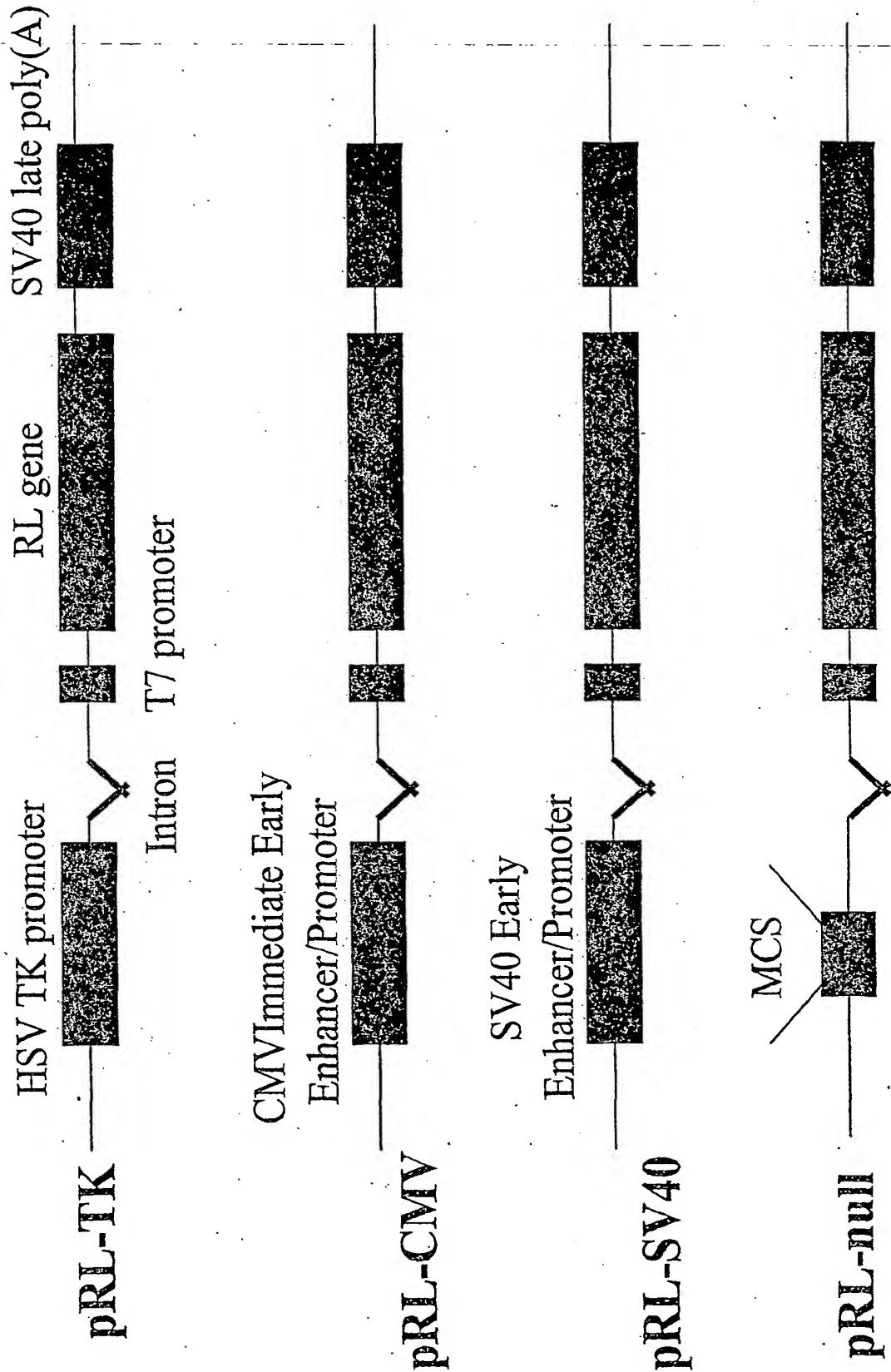
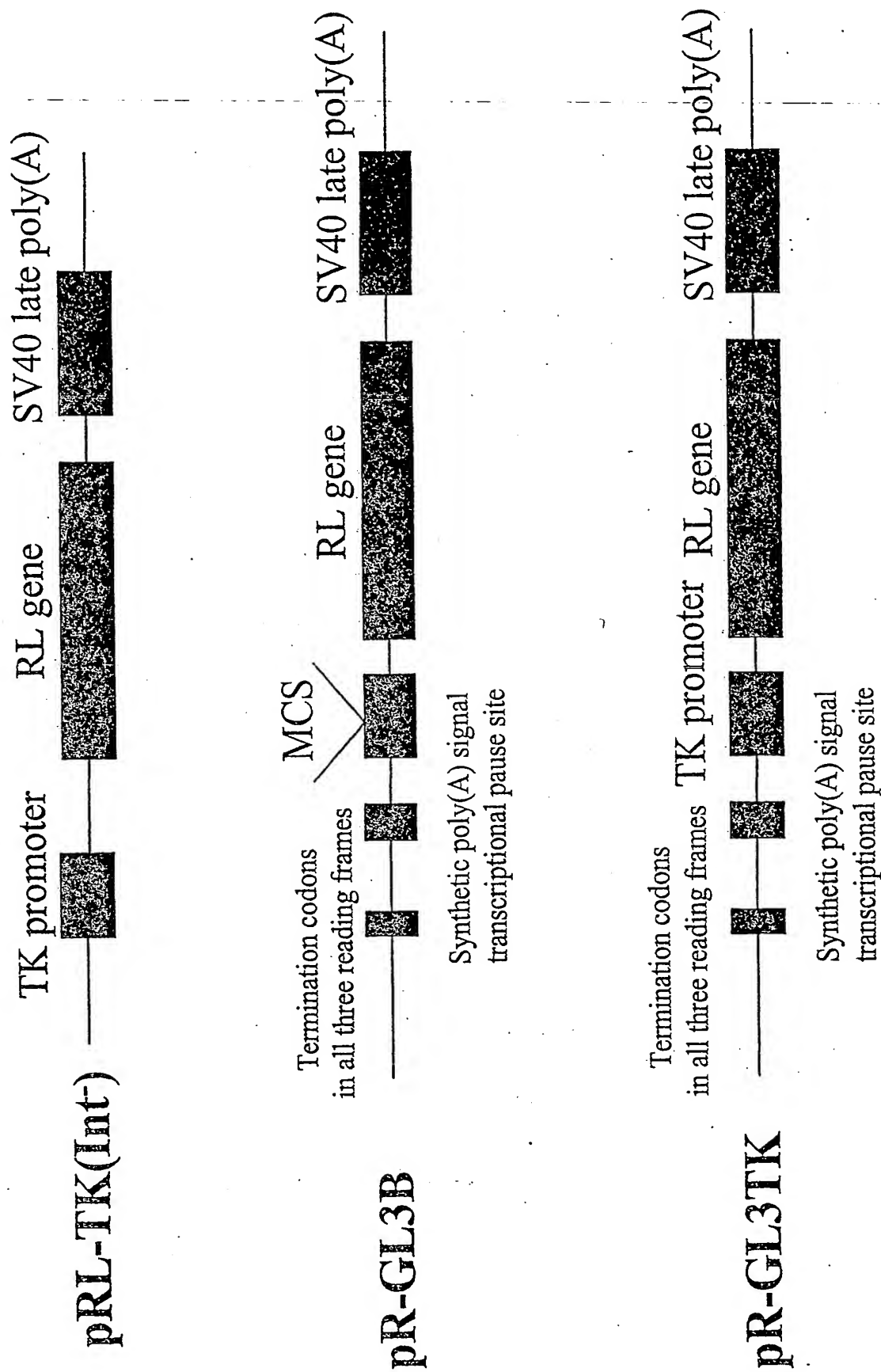


Fig 13A

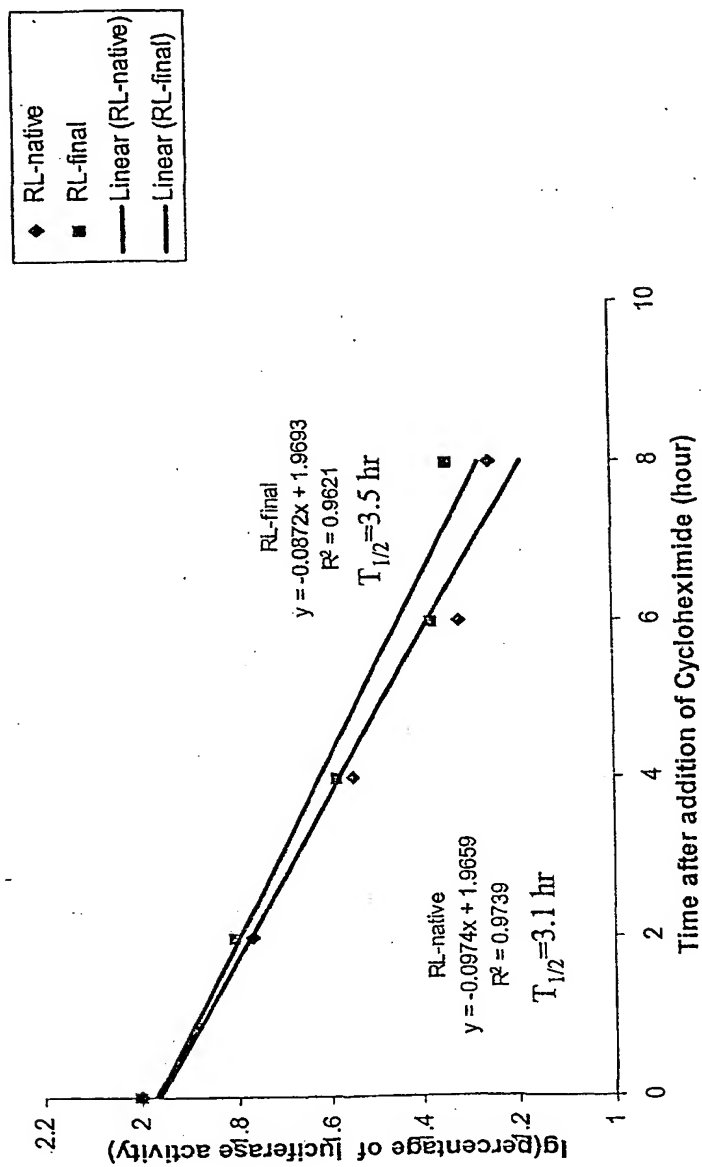
**Figure 13<sup>B</sup>- RL Co-Reporter Vector Series**



<sup>B</sup>  
**Figure 13 (Continued)**

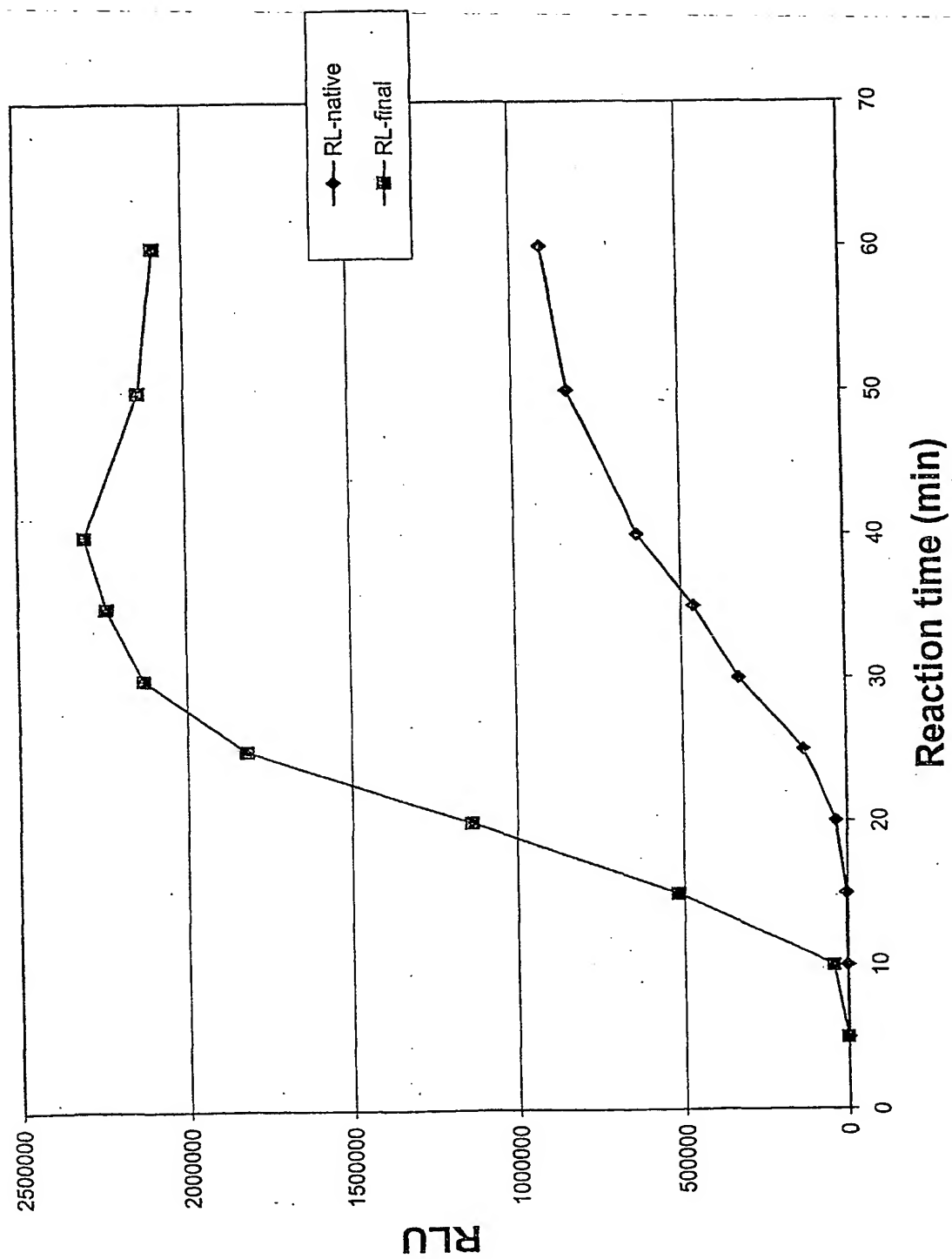


## Half-life of RL-synthetic and RL-native in CHO Cells

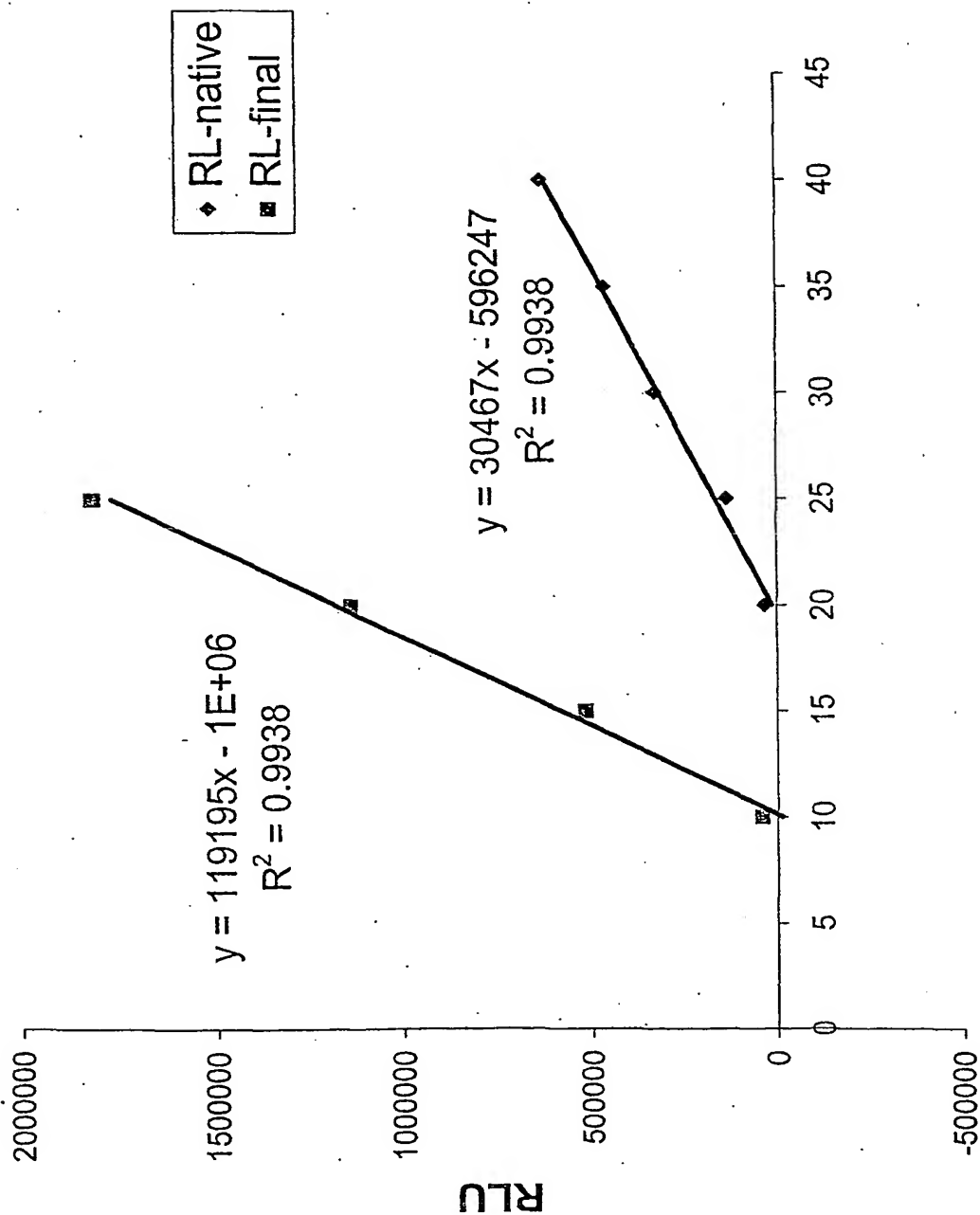




## TNT (RL-final versus RL-native)

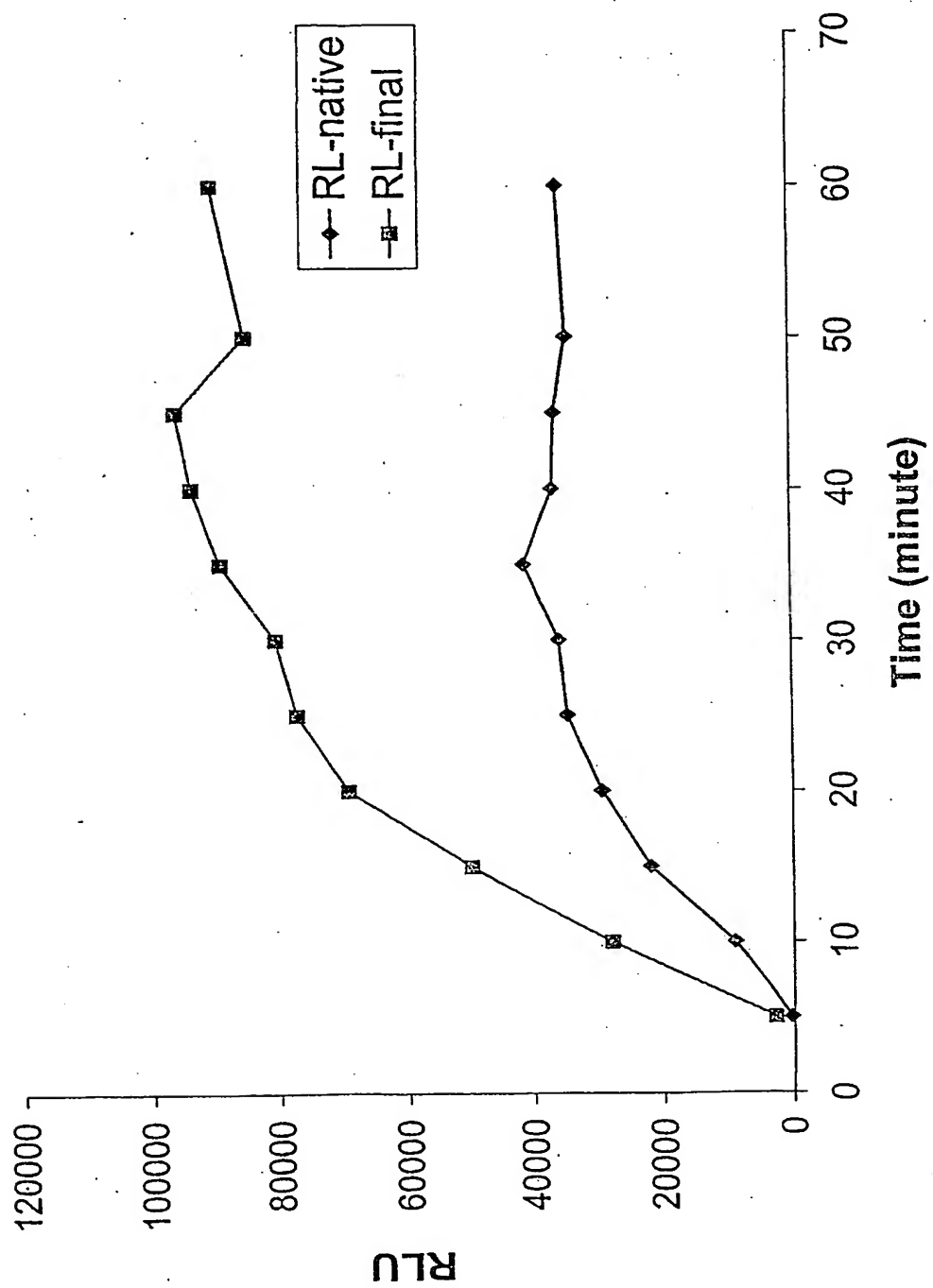


TNT (RL-final versus RL-native, linear range)

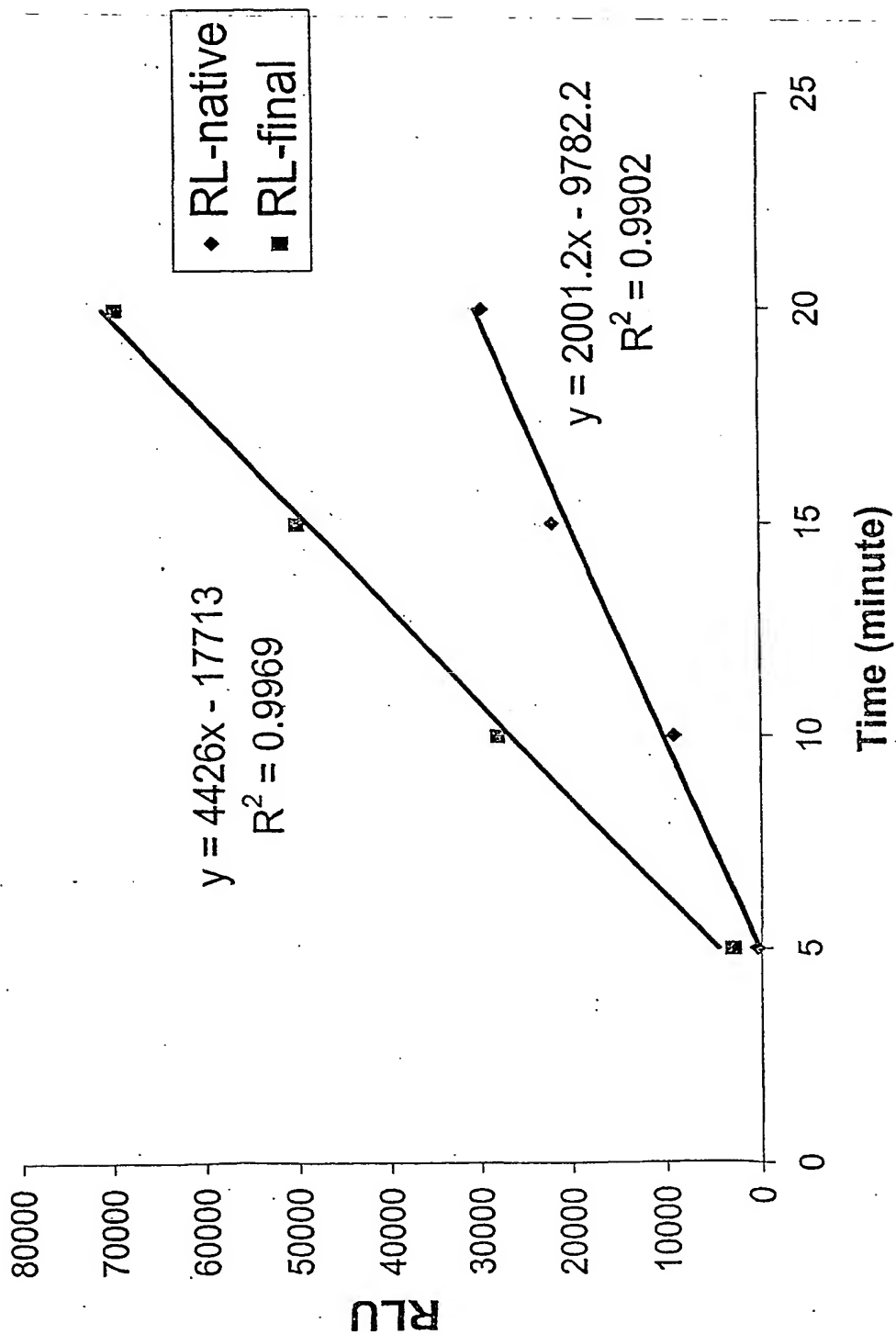


Reaction time (minutes)

# In vitro translation of RNAs of native RL and RL-final (30°C)

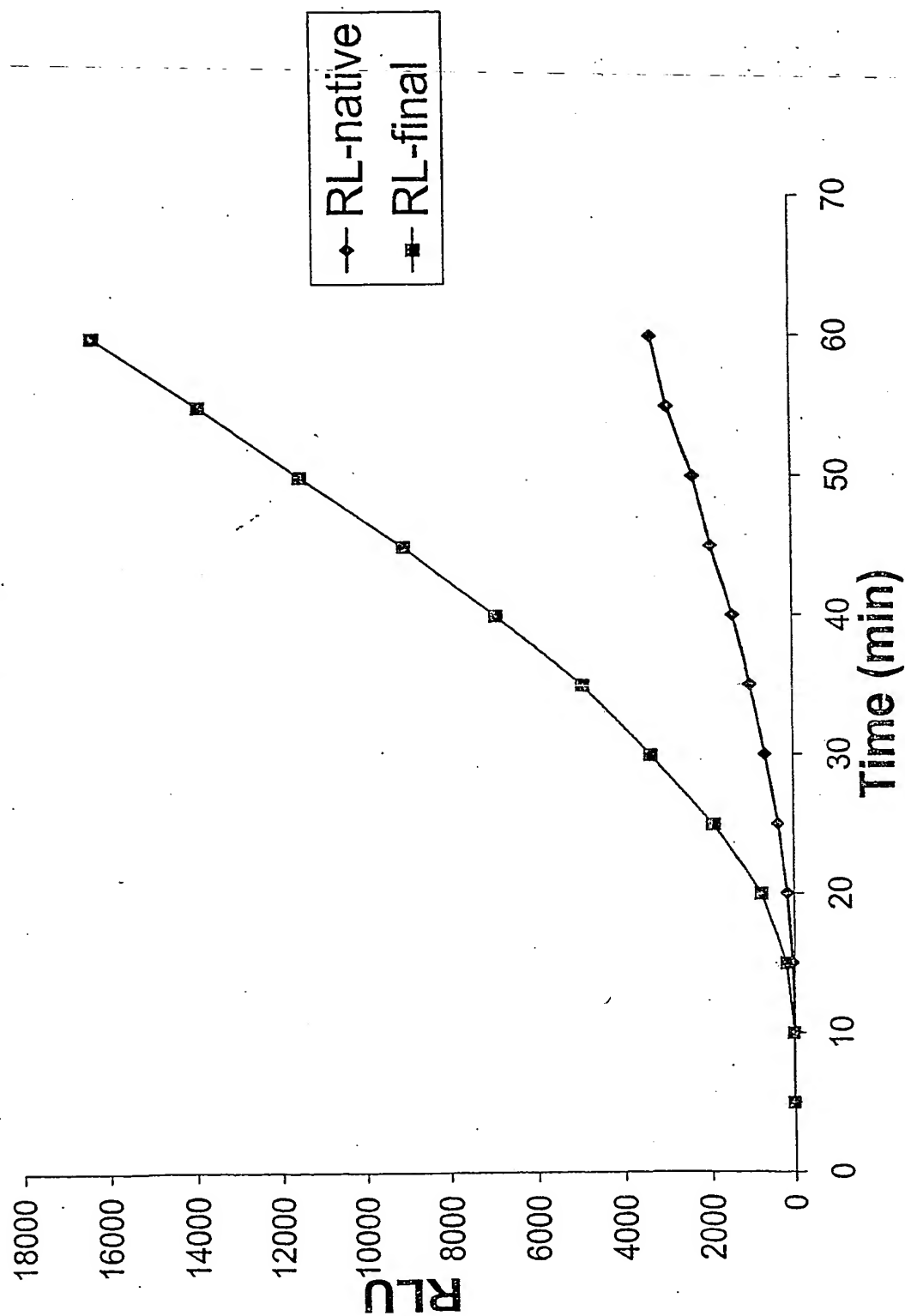


**In vitro translation of RNAs of native RL and RL-final  
(30 °C, linear range)**

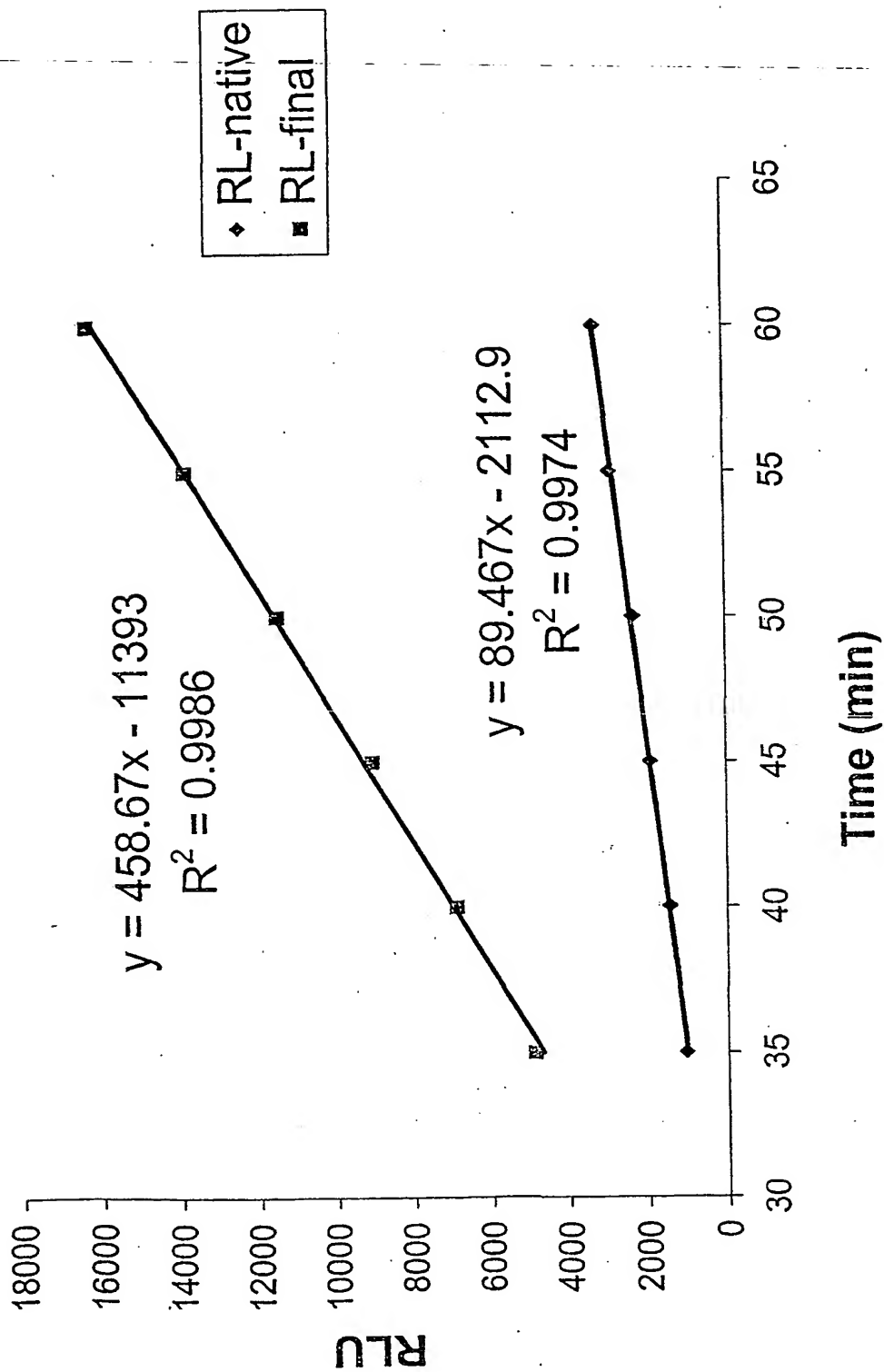


# In vitro translation using wheat germ

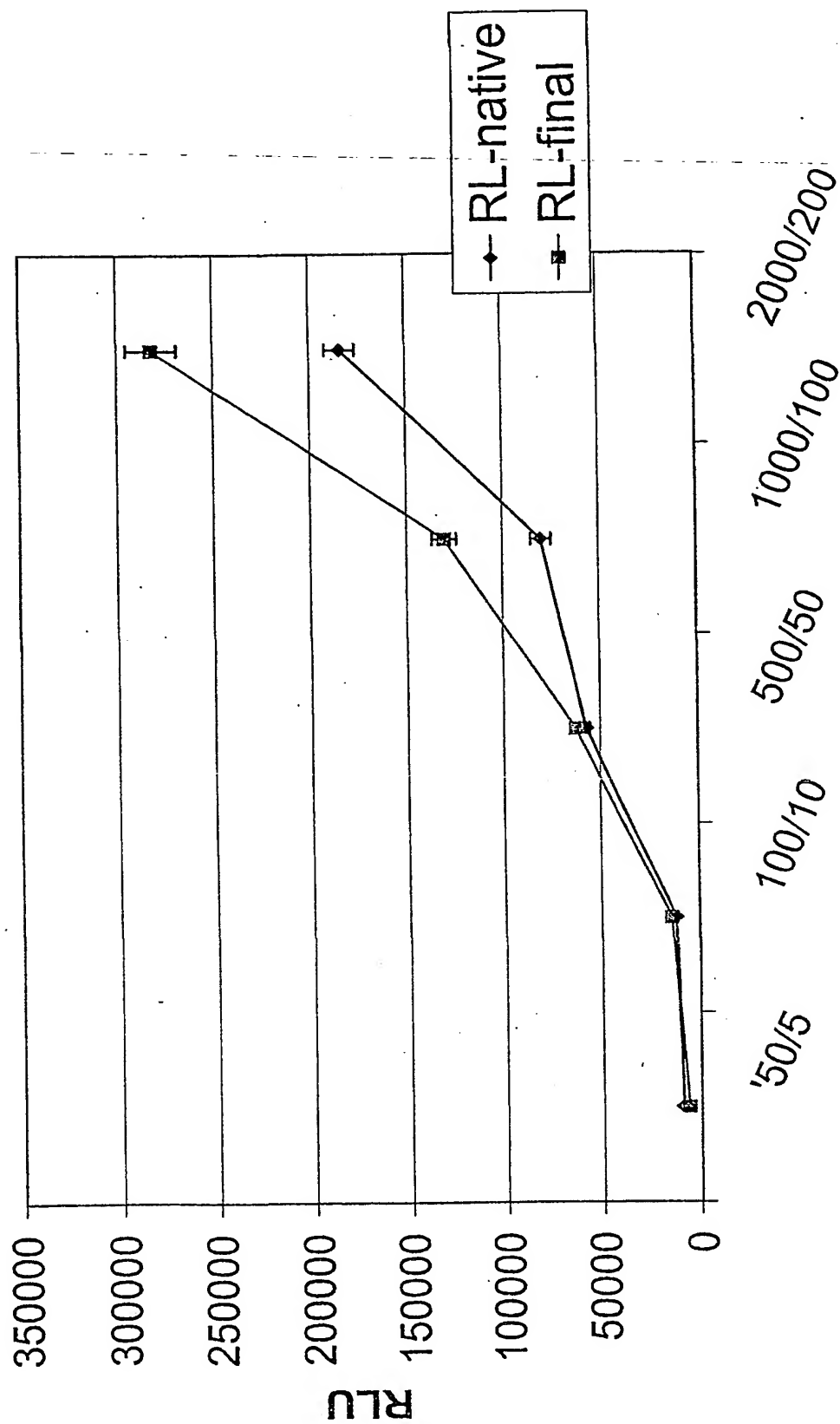
extract



# In vitro translation using wheat germ extract (linear range)



# Renilla expression



ng Renilla vector co-transfected (nat./final)

# Effect of firefly expression with increasing amounts of TK vector co-transfected

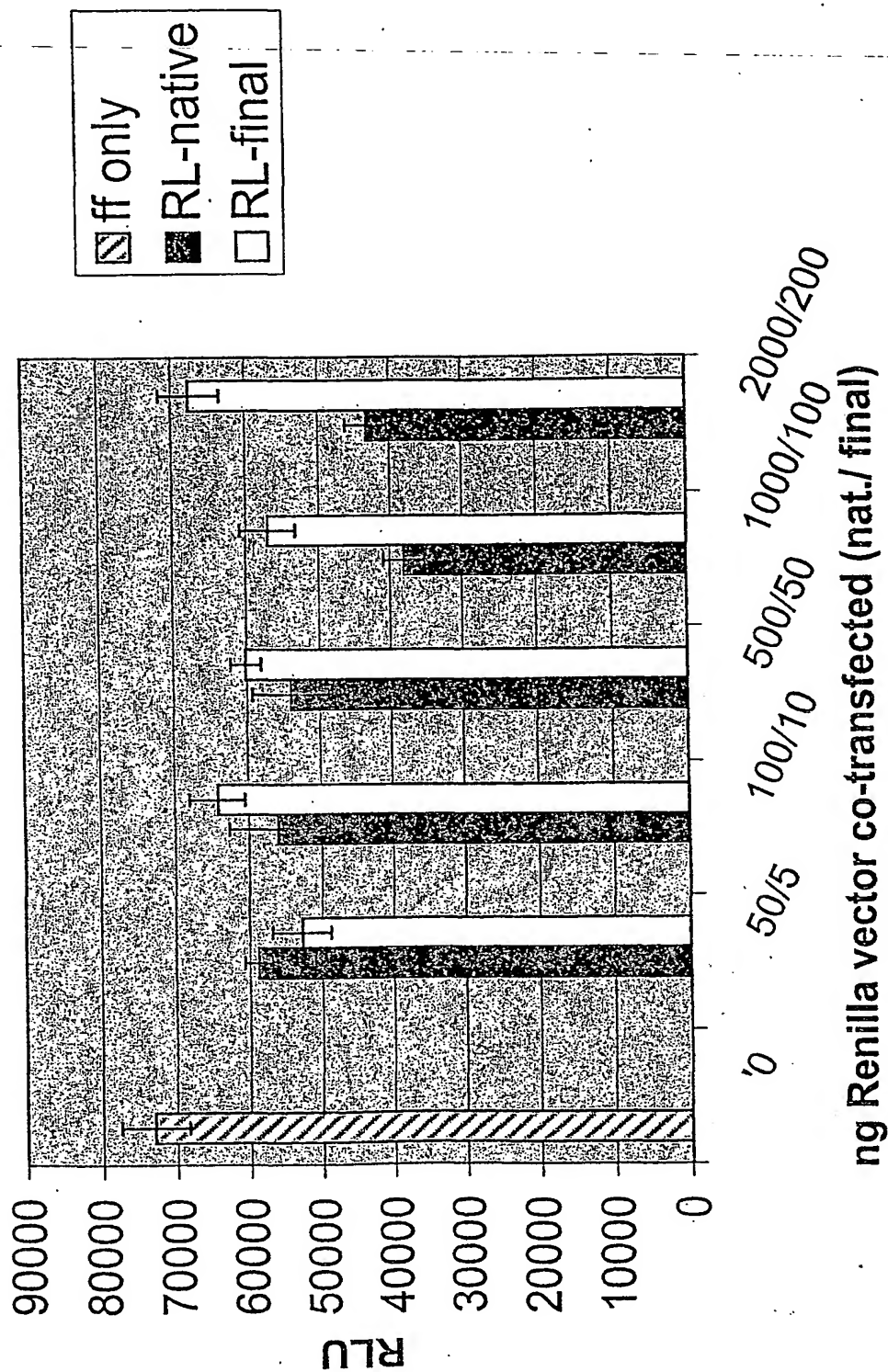




Figure 17 A

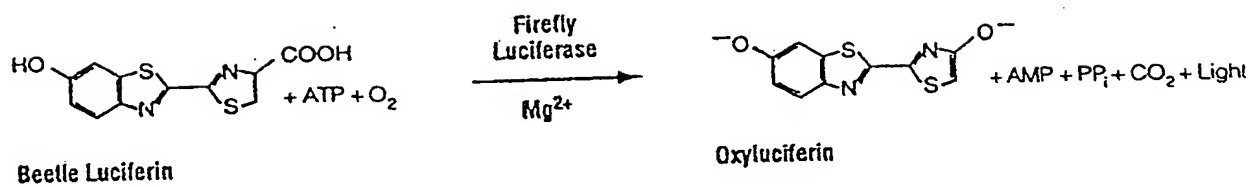
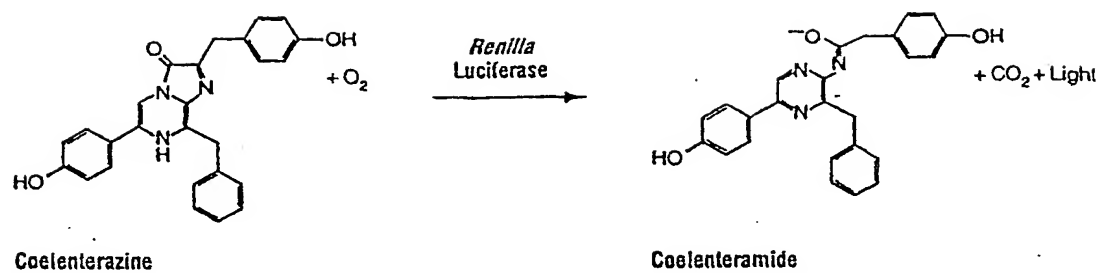


Figure 2 173



## GRver5.1 DNA sequence of pGL3 vectors

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GTCGCCCCCTGTGAATGAATCTTACATCCCAGATGAGCTGTGTAAGGTTAT 350
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CAAAGAAGTGGCCGAGGTCTGCTAAGCGTCTGAACCTCCCTGGTATCC 1000
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GGGTATCCCAGACTTGGAAGCTGGCGAGTTGCCTAGCGCCTTTGTGGTGA 1450
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GAGCGCGTGTCTCACACCAAATATCTGCGTGGCGGCGTCCGCTTCGTCTGA 1550
TTCTATTCCACGCAACGTTACCGGTAAGATCACTCGTAAAGAGTTGCTGA 1600
AGCAACTCCTCGAAAAAGCTGGCGGC 1626
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SEQ ID NO: 297

## RDver5.1 DNA sequence of pGL3 vectors

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CCATGCTCTCGATCCACGCTACGGCACTCAGCTGATTCTTGGTGTCAACG 700
TCTTGGTCTACTTGCCTTTCTTCCATGCTTTTCGGCTTTTCATATTACTTTG 750
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GGAGGCCTTTCTTGAAAGCCATCCAAGATTATGAAGTCCGCAGTGTATCA 850
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SEQ ID NO: 299

## RD1561H9 DNA sequence of pGL3 vectors

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SEQ ID NO. 301

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LPKGVMTQTHQNICVRLIHALDPRVGTQLIPGVTVLVYLPFFHAFGFSITL 250  
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ERVSHTKYLRRGGVRFVDSIPRNVTKITRKELLKQLLEKAGG 542

SEQ ID NO: 298

**RDver5.1 protein sequence of pGL3 vectors**

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SEQ ID NO: 300

**RD1561H9 protein sequence of pGL3 vectors**

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LDTVENIHGCESLPNFISRYSDGNIANFKPLHFDPEQVAAILCSSGTTG 200  
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## SEQUENCE LISTING

<110> Promega Corporation

5 Wood, Keith V.

Gruber, Monika G.

Zhuang, Yao

Paguio, Aileen

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| gccgagaata ataaaagatt ttttattccc attattgcag cttggtatat tggatatgatt   | 300 |
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| aaaccacaaa tagttttttg tacaaagaac atttttaaata aggtattgga ggtacagagc   | 420 |
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| gaaagtcttc ccaattttat ttctcggttat tcggatggaa atattgcca cttcaaacct    | 540 |
| ttacattacg atcctggtga gcaagtggca gctatcttat gttcgtcagg cactactgga    | 600 |
| ttaccgaaag gtgtaatgca aactcaccaa aatatttgtg tccgacttat acatgottta    | 660 |
| gacccaggg caggaacgca acttattcct ggtgtgacag tcttagtata tctgcctttt     | 720 |
| 40ttccatgctt ttgggttctc tataaacttg ggatacttca tgggtgggtct tcgtgttato | 780 |
| atgttaagac gatttgatca agaagcattt ctaaaagcta ttcaggatta tgaagttcga    | 840 |

```

agtgttaatta acgttccagc aataatattg ttcttatcga aaagtccttt ggttgacaaa      900
tacgatttat caagtttaag ggaatttgtgt tgcggtgcgg caccattagc aaaagaagtt      960
getgaggttg cagtaaacg attaaacttg ccaggaattc gctgtggatt tggtttgaca      1020
gaatctactt cagctaatat acacagtcct ggggatgaat ttaaatcagg atcacttgga      1080
5agagttactc ctttaatggc agctaaaata gcagataggg aaactggtaa agcattggga      1140
ccaaatcaag ttggtgaatt atgcgttaaa ggtcccatgg tatcgaaagg ttacgtgaac      1200
aatgtagaag ctaccaaaga agctattgat gatgatgggt ggcttcactc tggagacttt      1260
ggatactatg atgaggatga gcatttctat gtggtggacc gttacaagga attgattaaa      1320
tataagggct ctcaggtagc acctgcagaa ctagaagaga ttttattgaa aaatccatgt      1380
10atcagagatg ttgctgtggt tggatttcct gatctagaag ctggagaact gccatctgcg      1440
tttgtgggta aacagcccgg aaaggagatt acagctaaag aagtgtacga ttatcttgcc      1500
gagaggggtct cccatacaaa gtatttgctg ggaggggttc gattcgttga tagcatacca      1560
aggaatgtta caggtaaaat tacaagaaag gaacttctga agcagttgct ggagaagagt      1620
tctaaactt                                     1629

```

15

&lt;210&gt; 2

&lt;211&gt; 1626

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

20

&lt;220&gt;

&lt;223&gt; Sequence of clone YG#81-6G01

&lt;400&gt; 2

```

25atgatgaagc gagagaaaaa tggtatatat ggaccogaac ccctacaccc cttggaagac      60
ttaacagctg gagaaatgct cttccgtgcc cttcgaaaac attctcattt accgcaggct      120
ttagtagatg tgggtggcga cgaatcgctt tcctataaaag agttttttga agcgacagtc      180
ctcctagcgc aaagtctcca caattgtgga tacaagatga atgatgtagt gtgcgatctgc      240
gccgagaata atacaagatt ttttattccc gttattgcag cttggtatat tggatatgatt      300
30tagcacctg ttaatgaaag ttacatccca gatgaactct gtaaggatgat gggatatatcg      360
aaaccacaaa tagttttttac gacaaagaac atttttaaata aggtattgga ggtacagagc      420
agaactaatt tcataaaaag gatcatcata cttgatactg tagaaaacat acacggttgt      480
gaaagtcttc ccaattttat ttctcgttat tcggatggaa atattgccaa cttcaaacct      540
ttacatttcg atcctgttga gcaagtggca gctatcttat gttcgtcagg cactactgga      600
35ttaccgaaag gtgtaatgca aactcaccaa aatatttgtg tccgacttat acatgcttta      660
gacccagggg caggaacgca acttattcct ggtgtgacag tcttagtata tctgcctttt      720
ttccatgctt ttgggttctc tataaccttg ggatacttca tgggtgggtct tcgtgttatc      780
atgttcagac gatttgatca agaagcattt ctaaaagcta ttcaggatta tgaagttcga      840
agtgttaatta acgttccatc agtaatattg ttcttatcga aaagtccttt ggttgacaaa      900
40tacgatttat caagtttaag ggaatttgtgt tgcggtgcgg caccattagc aaaagaagtt      960

```



3

gctgaggttg cagcaaaacg attaaacttg ccaggaattc gctgtggatt tggtttgaca 1020  
 gaatctactt cagctaatat acacagtctt agggatgaat ttaaatcagg atcacttgga 1080  
 agagttactc ctttaatggc agctaaaata gcagataggg aaactggtaa agcattggga 1140  
 ccaaatacaag ttggtgaatt atgcattaaa ggtcccatgg tatcgaaagg ttacgtgaac 1200  
 5aatgtagaag ctaccaaaga agctattgat gatgatgggt ggcttcactc tggagacttt 1260  
 ggatactatg atgaggatga gcatttctat gtgggtggacc gttacaagga attgattaaa 1320  
 tataagggct ctcaggtagc acctgcagaa ctagaagaga ttttattgaa aaatccatgt 1380  
 atcagagatg ttgctgtgggt tggatttcct gatctagaag ctggagaact gccatctgcy 1440  
 tttgtgggta aacagcccgg aaaggagatt acagctaaag aagtgtacga ttatcttgcc 1500  
 10gagaggggtc cccatacaaa gtatttgogt ggagggggtc gattcggtga tagcatacca 1560  
 aggaatgtta caggtaaaat tacaagaaag gaacttctga agcagttgct ggagaaggcg 1620  
 ggaggt 1626

&lt;210&gt; 3

15&lt;211&gt; 1626

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

20&lt;223&gt; Sequence of a synthetic luciferase

&lt;400&gt; 3

atgatgaaac gcgaaaagaa cgctcatctac ggcccagagc ctctgcaccc attggaagac 60  
 ctgaccgccg gtgagatggt gttccgtgct ctgcgtaaac attctcactt gcctcaagcc 120  
 25ctgggtggatg tcgtgggoga cgaaagcttg tcttataagg agtttttcga agctactgtc 180  
 ctgttggccc agtctctgca taattgcggt taaaaaatga acgatgtgggt cagcatttgt 240  
 gctgagaata acacccgctt tttcatccca gtgattgccg cttggtacat cggcatgatt 300  
 gtcgcccttg tgaatgaatc ttatatccca gacgagttgt gcaaggatcat gggattatgc 360  
 aaacctcaaa tcgtgtttac taccaagaac attctgaata aagtcttggga agtgcagtct 420  
 30cgtactaact tcatcaagcg cattatcatt ctggataccg tcgagaatat ccacggctgt 480  
 gaaagcttgc caaactttat ttctcggtat agcgacggta atatcgctaa cttcaagcct 540  
 ctgcattttg atccagtggg gcaagtcgcc gctattttgt gctctagcgg cactaccggt 600  
 ctgcctaaag gcgtgatgca gactcaccaa aatatctgtg tccgcttgat tcatgccctg 660  
 gaccacgtg tgggtaccca gttgatccct ggctgactg tccgtgtgta cttgccatcc 720  
 35tttcacgcct tcggtttttc tattaccctg ggctatttca tgggtcggttt gcgcgtgatc 780  
 atgtttcgtc gcttcgatca agaagctttt ctgaaggcca ttcaggacta cgaggtccgt 840  
 agcgtgatca acgtcccttc tgtgattttg ttccgtagca aatctccatt ggtcgataag 900  
 tatgacctga gctctttgcy cgaactgtgc tgtggcgctg cccctttggc taaagaggtg 960  
 gccgaagtgc ctgccaagcg tctgaatttg ccaggatatcc gctgcggcctt tgggtctgact 1020  
 40gagagcacct ctgctaaccat tcatagcttg cgtgatgaat tcaaatctgg cagcctgggt 1080

|             |            |            |             |            |            |      |
|-------------|------------|------------|-------------|------------|------------|------|
| cgcggtgactc | ctttgatggc | cgctaagatc | gccgaccgtg  | agaccggcaa | agctctgggt | 1140 |
| ccaaatcaag  | tcggcgaatt | gtgtattaag | ggtcctatgg  | tgtctaaagg | ctacgtcaac | 1200 |
| aatgtggagg  | ccactaagga | agctatcgat | gacgatgggt  | ggctgcacag | cggcgacttt | 1260 |
| ggttattacg  | atgaggacga | acatttctat | gtcgtggatc  | gctacaaaga | gttgattaag | 1320 |
| 5tataaaggct | ctcaggtcgc | cccagctgag | ctggaagaga  | tcttgctgaa | gaacccttgc | 1380 |
| attcgtgacg  | tggccgtcgt | gggtatccca | gatttggaag  | ctggcgagct | gcctagcgcc | 1440 |
| tttgtcgtga  | aacaaccagg | taaggaaatt | accgctaaag  | aggtctacga | ctatttggcc | 1500 |
| gaacgcgtgt  | ctcacactaa | gtacctgcgt | ggcgggtgtcc | gcttcgtgga | tagcatccct | 1560 |
| cgcaatgtca  | ccggcaaaat | tactcgtaag | gagttgctga  | aacagttgct | ggaaaaggct | 1620 |
| 10gggtggc   |            |            |             |            |            | 1626 |

&lt;210&gt; 4

&lt;211&gt; 1626

&lt;212&gt; DNA

15&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Sequence of a synthetic luciferase

20&lt;400&gt; 4

|              |             |             |             |             |             |      |
|--------------|-------------|-------------|-------------|-------------|-------------|------|
| atgatgaaac   | gcgaaaagaa  | cgctcatctac | ggcccagagc  | ctctgcaccc  | attggaagac  | 60   |
| ctgaccgctg   | gtgagatggt  | gttccgtgct  | ctgcgtaaac  | attctcactt  | gcctcaagcc  | 120  |
| ctggtcgatg   | tcgtggggca  | cgagagcttg  | tcttataagg  | aatttttcga  | agctactgtc  | 180  |
| ctggttgccc   | aatctctgca  | taattgcggt  | tacaaaatga  | acgatgtggg  | cagcatttgt  | 240  |
| 25gctgagaata | acaccgcctt  | tttcatccca  | gtgattgccg  | cttggtacat  | cggcattgatt | 300  |
| gtcgcccctg   | tgaatgaatc  | ttatatccca  | gacgagttgt  | gcaaggcat   | gggtattagc  | 360  |
| aaacctcaaa   | tcgtgtttac  | taccaagaac  | attctgaata  | aggtcttgga  | agtgcagtct  | 420  |
| cgtaactaact  | tcataaagcg  | cattatcatt  | ctggataccg  | tcgagaatat  | ccacggctgt  | 480  |
| gagagcttgc   | caaactttat  | ttctcgttat  | agcgacggta  | atatcgctaa  | cttcaagcct  | 540  |
| 30ctgcattttg | atccagtggg  | gcaagtgcgc  | gctattttgt  | gctctagcgg  | caccaccggt  | 600  |
| ctgcctaaag   | gcgtgatgca  | gaotcaccac  | aatatctgtg  | tccgcttgat  | tcattgcctg  | 660  |
| gaccacgctg   | tgggtactca  | gttgatccct  | ggcgtgactg  | tcctgggtga  | cttgccattc  | 720  |
| tttcacgcct   | tcgggtttttc | tattaccctg  | ggctatttca  | tggtcgggtt  | gcgcgtgact  | 780  |
| atgtttcgtc   | gcttcgatca  | agaagccttt  | ctgaaggcca  | ttcaagacta  | cgagggtccgt | 840  |
| 35agcgtgatca | acgtcccttc  | tgtgattttg  | ttcctgagca  | aatctccatt  | ggctcgataag | 900  |
| tatgacctga   | gcagcttgcg  | cgaactgtgc  | tgtggcgctg  | cccctttggc  | taaagagggtg | 960  |
| gccgaagtgc   | ctgccaaagcg | tctgaatttg  | ccaggatatcc | gctgcggctt  | tgggtctgact | 1020 |
| gagagcacct   | ctgctaacat  | tcatagcttg  | cgtgatgagt  | tcaaactctgg | cagcctgggt  | 1080 |
| cgcggtgactc  | ctttgatggc  | cgctaagatc  | gccgaccgtg  | agaccggcaa  | agctctgggt  | 1140 |
| 40ccaaatcaag | tcggcgaatt  | gtgtattaag  | ggtcctatgg  | tgtctaaagg  | ctacgtcaac  | 1200 |

5

|             |            |            |             |            |            |      |
|-------------|------------|------------|-------------|------------|------------|------|
| aatgtggagg  | ccactaagga | agctattgat | gacgatgggt  | ggctgcacag | cggcgacttt | 1260 |
| ggttattacg  | atgaggacga | acatttctat | gtcgtcgatc  | gctacaaaga | gttgattaag | 1320 |
| tataaaggct  | ctcaagtcgc | cccagctgag | ctggaagaaa  | tcttgctgaa | gaacccttgc | 1380 |
| attcgtgacg  | tggccgtcgt | gggtatccca | gatttggaag  | ctggcgagct | gcctagcgcc | 1440 |
| 5tttgtcgtga | aacaaccagg | caaggaaatt | accgctaaag  | aggtctacga | ctatttggcc | 1500 |
| gagcgcgtgt  | ctcacactaa | gtacctgcgt | ggcgggtgtcc | gcttcgtcga | tagcatccct | 1560 |
| cgcaatgtca  | cgggcaaaat | tactcgtaag | gagttgctga  | aacagttgct | ggaaaaggct | 1620 |
| ggtggc      |            |            |             |            |            | 1626 |

10&lt;210&gt; 5

&lt;211&gt; 1626

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

15&lt;220&gt;

&lt;223&gt; Sequence of a synthetic luciferase

&lt;400&gt; 5

|              |             |             |             |             |             |      |
|--------------|-------------|-------------|-------------|-------------|-------------|------|
| atgatgaaac   | gcgaaaagaa  | cgtgatctac  | ggcccagAAC  | cactgcatcc  | actggaagac  | 60   |
| 20ctcaccgctg | gtgagatgct  | gttcogtgcc  | ctgcgtaaac  | atagccacct  | gcctcaagct  | 120  |
| ctcgtggacg   | tcggtgggtga | cgagagcctg  | tcttaciaag  | aatttttcga  | agctactgtg  | 180  |
| ctgttggccc   | aaagcctgca  | taattgtggt  | tacaaaatga  | acgatgtggt  | gagcatctgt  | 240  |
| gctgagaata   | acactcgctt  | ttttatccct  | gtgatcgctg  | cttggtacat  | cggcattgatt | 300  |
| gtcgccccctg  | tgaatgaatc  | ttacatccca  | gatgagttgt  | gtaaggatgat | gggtattagc  | 360  |
| 25aaacctcaaa | tcgtcttttac | tacaaaaaac  | atcctgaata  | aggtcttgga  | agtccagtct  | 420  |
| cgtactaatt   | tcatcaaacg  | cattattatt  | ctggataccg  | tcgaaaacat  | ccacggctgt  | 480  |
| gagagcttgc   | ctaactttat  | ctctcggttac | agcgatggta  | atatcgctaa  | tttcaagcca  | 540  |
| ctgcattttg   | atccagtcga  | gcaggtcgcc  | gccattttgt  | gctcttctgg  | caccactggg  | 600  |
| ttgcctaaag   | gtgtcatgca  | gactcaccag  | aatatctgtg  | tgcgcttgat  | ccacgccctc  | 660  |
| 30gaccctcgtg | tgggtactca  | attgatccct  | ggcgtgactg  | tgcgtggtga  | tttgcccttc  | 720  |
| tttcacgcct   | ttgggtttttc | tatcaccctg  | ggctattttca | tggtcgggctt | gcgtgtgac   | 780  |
| atgtttcgtc   | gcttcgacca  | agaagccttc  | ctgaaggcta  | ttcaagacta  | cgagggtcgt  | 840  |
| tctgtgatca   | atgtcccatc  | tgatcattttg | ttcctgagca  | aatctccttt  | ggttgacaag  | 900  |
| tatgatctga   | gcagcttgcg  | tgaactgtgc  | tgtggcgctg  | ctcctttggc  | caaagaagtg  | 960  |
| 35gccgaggctg | ctgctaagcg  | tctgaacctc  | cctggatatcc | gctgcgggttt | tgggttgact  | 1020 |
| gagagcactt   | ctgccaacat  | ccatagcttg  | cgtgacgagt  | ttaaatctgg  | tagcctgggt  | 1080 |
| cgcgtgaccc   | ctttgatggc  | tgcaaagatc  | gccgaccgtg  | agaccggcaa  | agccctgggc  | 1140 |
| ccaaatcagg   | tcggtgaatt  | gtgcattaag  | ggccctatgg  | tctctaaagg  | ctacgtgaac  | 1200 |
| aatgtggagg   | ccactaaaga  | agctattgat  | gatgatgggt  | ggttgcatag  | cggcgacttc  | 1260 |
| 40ggttattatg | atgaggacga  | acacttctat  | gtggtcgac   | gctataaaga  | attgattaag  | 1320 |

6

|   |      |
|---|------|
| tacaaaggct ctcaagtcgc cccagctgaa ctggaagaaa ttttgctgaa gaacccttgt   | 1380 |
| attcgcgcagc tggccgtcgt gggatatccca gacttggaag ctggcgagtt gcctagcgcc | 1440 |
| tttggtgga aacaacctgg caaggagatt actgctaagg aggtctacga ctatttgccc    | 1500 |
| gagcgcgtgt ctcacactaa atatctgcgt ggcggcgctcc gcttcgtcga ttctatccct  | 1560 |
| 5cgcaacgtca ccggcaagat cactcgtaaa gagttgctga aacaattgct cgaaaaagct  | 1620 |
| ggcggc  | 1626 |

&lt;210&gt; 6

&lt;211&gt; 1626

10&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Sequence of a synthetic luciferase

15

&lt;400&gt; 6

|   |      |
|---|------|
| atgatgaaac gcgaaaagaa cgtgatctac ggcccagaac cactgcatcc actggaagac     | 60   |
| ctcaccgctg gtgagatgct cttccgtgca ctgcgtaaac atagtcacct ccctcaagct     | 120  |
| ctcgtggacg tcgtgggaga cgagagcctc tcttaciaaag aatttttcga agctactgtg    | 180  |
| 20ctgtttggccc aaagcctcca taattgtgga taaaaaatga acgatgtggg gagcatttgt  | 240  |
| gctgagaata aactcgcctt ctttatccct gttatcgtg cttgggtacat cggcatgatt     | 300  |
| gtcgcgccctg tgaatgaatc ttacatccca gatgagctgt gtaagggtat gggatttagc    | 360  |
| aaacctcaaa tcgtctttac taccaaaaat atcctgaata aggtccttga agtccagtct     | 420  |
| cgtactaact tcatcaaacg catcattatt ctggataccg tcgaaaacat ccatggctgt     | 480  |
| 25gagagcctgc ctaacttcat ctctcgttac agcgatggta atatcgctaa tttcaaacca   | 540  |
| ctgcattttg atccagtcga gcaagtggcc gctattttgt gctcttccgg caccactggg     | 600  |
| ttgcctaaag gtgtcatgca gactcaccag aatatctgtg tgcgtttgat ccacgctctc     | 660  |
| gaccctcgtg tgggtactca attgatccct ggcgtgactg tgctgggtga tctgcctttc     | 720  |
| tttcacgcct ttggtttttc tattaccctg ggctatttca tggtcggcct gcgtgtcatc     | 780  |
| 30atgtttcgtc gcttcgacca agaagccttc ttgaaggcta ttcaagacta cgaggtgcgt   | 840  |
| tctgtcatca atgtcccttc agtcattttg ttcctgagca aatctccttt ggttgacaag     | 900  |
| tatgatctga gcagcttgog tgagctgtgc tgtggcgctg ctcccttggc caaagaagtg     | 960  |
| gccgaggtcg ctgctaagcg tctgaacctc cctggatatcc gctgcgggtt tggtttgact    | 1020 |
| gagagcactt ctgctaacat ccatagcttg cgagacgagt ttaagtctgg tagcctgggt     | 1080 |
| 35cgcgtagctc ctcttatggc tgcaaaagatc gccgaccgtg agaccggcaa agcactgggc  | 1140 |
| ccaaatcaag tcggtgaatt gtgtattaag ggccctatgg tctctaaagg ctacgtgaac     | 1200 |
| aatgtggagg ccactaaaga agccattgat gatgatggct ggctccatag cggcgacttc     | 1260 |
| ggttactatg atgaggacga acacttctat gtggtcgacg gctacaaaga attgattaag     | 1320 |
| tacaaaggct ctcaagtcgc cccagccgaa ctggaagaaa ttttgctgaa gaacccttgt     | 1380 |
| 40atccgcgacg tggccgtcgt gggatatccca gacttggaag ctgggtgagtt gcctagcgcc | 1440 |

|  |      |
|--|------|
| tttgtggtga aacaacctgg aaaggagatc actgctaagg aggtctacga ctatttggcc  | 1500 |
| gagcgcgtgt ctcacaccaa atatctgcgt ggcggcgtcc gcttcgtcga ttccatccca  | 1560 |
| cgcaacgtga ccggtgaagat cactcgtaaa gaattgctga agcaactcct cgaaaaagct | 1620 |
| ggcggc   | 1626 |

5

&lt;210&gt; 7

&lt;211&gt; 1626

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

10

&lt;220&gt;

&lt;223&gt; Sequence of a synthetic luciferase

&lt;400&gt; 7

|  |      |
|--|------|
| 15atgatgaaac gcgaaaagaa cgtgatctac ggcccagaac cactgcatcc actggaagac  | 60   |
| ctcaccgctg gtgagatgct cttccgagca ctgcgtaaac atagtcacct ccctcaagca    | 120  |
| ctcgtggacg tcggtggaga cgagagcctc tcctacaaag aatttttcga agctactgtg    | 180  |
| ctggttggccc aaagcctcca taattgtggg tacaaaatga acgatgtggg gagcatttgt   | 240  |
| gctgagaata acactcgctt ctttattcct gtaatcgctg cttggtacat cggcatgatt    | 300  |
| 20gtcgcccctg tgaatgaatc ttacatccca gatgagctgt gtaagggttat gggatttagc | 360  |
| aaacctcaaa tcgtctttac taccaaaaac atcttgaata aggtcttgga agtccagtct    | 420  |
| cgtactaact tcatcaaagc catcattatt ctggataccg tcgaaaacat ccacggctgt    | 480  |
| gagagcctcc ctaacttcat ctctcggtac agcgatggta atatcgctaa tttcaagccc    | 540  |
| ttgcattttg atccagtoga gcaagtggcc gctattttgt gctcctccgg caccactggg    | 600  |
| 25ttgcctaaag gtgtcatgca gactcaccag aatatctgtg tgcgtttgat ccacgctctc  | 660  |
| gaccctcgtg tgggtactca attgatccct ggcggtgactg tgctggtgta tctgcctttc   | 720  |
| tttcacgcct ttggtttctc tattaccctg ggctatttca tggtcggcct gcgtgtcatc    | 780  |
| atgtttcgtc gcttcgacca agaagccttc ttgaaggcta ttcaagacta cgaggtcgt     | 840  |
| tccgtgatca acgtcccttc agtcattttg ttccctgagca aatctccttt ggttgacaag   | 900  |
| 30tatgatctga gcagcttgcg tgagctgtgc tgtggcgctg ctcccttggc caaagaagtg  | 960  |
| gccgaggtcg ctgctaagcg tctgaacctc cctggtatcc gctgcgggtt tggtttgact    | 1020 |
| gagagcactt ctgctaacat ccatagcttg cgagacgagt ttaagtctgg tagcctgggt    | 1080 |
| cgcgtgactc ctcttatggc tgcaaagatc gccgaccgtg agaccggcaa agcactgggc    | 1140 |
| ccaaatcaag tcggtgaatt gtgtattaag ggccctatgg tctctaaagg ctacgtgaac    | 1200 |
| 35aatgtggagg ccactaaaga agccattgat gatgatggct ggctccatag cggcgacttc  | 1260 |
| ggttactatg atgaggacga acacttctat gtggctgacg gctacaaaga attgattaag    | 1320 |
| tacaaaggct ctcaagtgcg accagccgaa ctggaagaaa ttttgctgaa gaaccttgt     | 1380 |
| atccgcgacg tggccgctgt gggtatccca gacttggaag ctggcgagtt gcctagcgcc    | 1440 |
| tttgtggtga aacaaccggg caaggagatc actgctaagg aggtctacga ctatttggcc    | 1500 |
| 40gagcgcgtgt ctcacaccaa atatctgcgt ggcggcgtcc gcttcgtcga ttctattcca  | 1560 |

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<210> 8

5<211> 1626

<212> DNA

<213> Artificial Sequence

<220>

10<223> Sequence of a synthetic luciferase

<400> 8

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15ctcgtggacg tcgtgggaga cgagaacctc tcctacaaag aatttttcga agctactgtg 180  
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&lt;210&gt; 9

&lt;211&gt; 1626

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

5

&lt;220&gt;

&lt;223&gt; Sequence of a synthetic luciferase

&lt;400&gt; 9

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&lt;210&gt; 10

40&lt;211&gt; 1626

10

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

5&lt;223&gt; Sequence of a synthetic luciferase

&lt;400&gt; 10

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35ggcgggt                                         1626

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&lt;210&gt; 11

&lt;211&gt; 1626

&lt;212&gt; DNA

40&lt;213&gt; Artificial Sequence



11

&lt;220&gt;

&lt;223&gt; Sequence of a synthetic luciferase

&lt;400&gt; 11

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  ggcggt                                           1626

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&lt;210&gt; 12

35&lt;211&gt; 1626

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

40&lt;223&gt; Sequence of a synthetic luciferase

12

<400> 12

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| ttggtcgatg tggtcggcga tgaatctttg agctataagg agtttttttga ggcaaccgtc  | 180  |
| 5ttgctggctc agtcttttgca taattgcggc tacaagatga acgacgtcgt ctctatttgt | 240  |
| gccgaaaaaca atacccggtt cttcattcca gtcacgcgcg cctggatatat cggtatgac  | 300  |
| gtggctccag tcaacgagag ctacattcct gacgaactgt gtaaagtcac gggatatctct  | 360  |
| aagccacaga ttgtgttcac cactaagaat attttgaaca aagtgcctgga agtccaaagc  | 420  |
| cgcaccaact ttattaagcg tatcatcato ttggacactg tggagaatat tcatgggtgc   | 480  |
| 10gaatctctgc ctaatttcat tagccgctat tctgacggca acatcgccaa ctttaaacct | 540  |
| ttgcatttgc accctgtgga acaagtggct gctatcctgt gtagcagcgg tactactggc   | 600  |
| ctcccaaagg gcgtcatgca gacccatcaa aacatttgcg tgcgtctgat ccatgctctc   | 660  |
| gatccacgct acggcactca gctgattcct ggtgtcaccg tcttgggtcta cctgcctttc  | 720  |
| ttccatgctt tcggcttcca cattactttg gggtacttta tggtcgggtct gcgtgtcatt  | 780  |
| 15atgttccgcc gttttgatca ggaggctttt ttgaaagcca tccaagatta tgaagtccgc | 840  |
| agcgtcatta acgtgcctag cgtgatcctg tttttgtcta agagccact cgtggacaag    | 900  |
| tacgacttgt ctccctgcg tgagtttgtt tgcggtgccg cccactggc taaggaggctc    | 960  |
| gctgaaagtgg ccgccaacg cttgaatctg ccaggcattc gttgtggctt cggcctcacc   | 1020 |
| gaatctacca gcgtattat tcaatctctc cgcgatgagt ttaagagcgg ctctttgggc    | 1080 |
| 20cgtgtcactc cactcatggc tgctaaaatc gctgatcgcg aaactggtaa ggctttgggc | 1140 |
| cctaaccaag tgggcgagct gtgtatcaaa ggccctatgg tgagcaaggg ttatgtcaat   | 1200 |
| aacgtcgaag ctaccaagga ggccatcgac gacgacggct ggctgcattc tggtgatttt   | 1260 |
| ggctactacg acgaagatga gcattttttac gtcgtggatc gttacaagga gctgatcaaa  | 1320 |
| tacaagggtg gccagggtggc tccagccgag ttggaggaga ttctgttgaa aaatccatgc  | 1380 |
| 25atccgtgatg tcgctgtggc cggcattcct gatctggagg ccggtgaact gccttctgct | 1440 |
| ttcgtcgtca agcagcctgg taaagaaatc accgccaag aagtgtatga ttacctggct    | 1500 |
| gaacgtgtga gccataccaa gtacttgcgt ggcgcgctgc gttttgtgga cagcattcca   | 1560 |
| cgtaatgtga ctggtaaaat taccgcgaag gaactgttga agcaattgtt ggagaaggcc   | 1620 |
| ggcggc  | 1626 |

30

&lt;210&gt; 13

&lt;211&gt; 1626

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

35

&lt;220&gt;

&lt;223&gt; Sequence of a synthetic luciferase

&lt;400&gt; 13

|   |    |
|---|----|
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|---|----|

|   |      |
|---|------|
| ttgactgccg gcgaaatgct gtttcgtgct ttgcgtaaac actctcattt gcctcaagcc   | 120  |
| ttggtcgatg tggtcggcga tgaatctttg agctacaagg agttttttga ggcaaccgtc   | 180  |
| ttgctggctc agtccttgca taattgtggc tacaagatga acgacgtcgt ctccatttgt   | 240  |
| gcagaaaaca ataccggtt cttcattcca gtcatgccg catggtatat cggtatgac      | 300  |
| 5gtggctccag tcaacgagag ctacattccc gacgaactgt gtaaagtcac gggatatctc  | 360  |
| aagccacaga ttgtcttcac cactaagaat attctgaaca aagtcctgga agtccaaagc   | 420  |
| cgcaccaact ttattaagcg tatcatcatc ttggacactg tggagaatat tcacggttgc   | 480  |
| gaatctttgc ctaattttat tagccgctat tcagacggaa acatcgccaa ctttaagcct   | 540  |
| ctccatttcg accctgtgga acaagttgct gcaatcctgt gtagcagcgg tactactgga   | 600  |
| 10ctcccaaagg gagtcatgca gacccatcaa aacatttgcg tgcgtctgat ccatgctctc | 660  |
| gatccacgct acggcactca gctgattcct ggtgtcaccg tcttggtcta cttgccttcc   | 720  |
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| agtgtcatca acgtgcctag cgtgatcctg tttttgtcta agagccact cgtggacaag    | 900  |
| 15tacgacttgt cttcactgcg tgaatttgtt tgcggtgccg ctccactggc taaggaggtc | 960  |
| gctgaagtgg ccgccaaacg cttgaatctg cccggcattc gttgtggctt cggcctcacc   | 1020 |
| gaatctacca gcgctattat tcagtctctc cgcgatgagt ttaagagcgg ctctttgggc   | 1080 |
| cgtgtcactc cactcatggc tgctaagatc gctgatcgcg aaactggtaa ggctttgggc   | 1140 |
| cctaaccaag tgggcgagct gtgtatcaaa ggccctatgg tgagcaaggg ttatgtcaat   | 1200 |
| 20aacgtcgaag ctaccaagga ggctatcgac gacgacggct gggtgcattc tggtgatttt | 1260 |
| ggatattacg acgaagatga gcattttttac gtcgtggatc gttacaagga gctgatcaaa  | 1320 |
| tacaagggta gccaggttgc tcagctgag ttggaggaga ttctgttgaa aaatccatgc    | 1380 |
| attcgcgatg tcgctgtggg cggcattcct gatctggagg ccggcgaaact gccttctgct  | 1440 |
| ttcgttgtca agcagcctgg taaagaaatt accgccaag aagtgtatga ttacctggct    | 1500 |
| 25gaacgtgtga gccatactaa gtacttgctg ggcgcgctgc gttttgtgga tagcattcct | 1560 |
| cgcaatgtga ctggcaaaat taccgcgaag gagctgttga aacaattgtt ggagaaggcc   | 1620 |
| ggcggg  | 1626 |

&lt;210&gt; 14

30&lt;211&gt; 1626

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

35&lt;223&gt; Sequence of a synthetic luciferase

&lt;400&gt; 14

|   |     |
|---|-----|
| atgatgaagc gtgagaaaaa tgtcatctat ggccctgagc ctctccatcc tttggaggat   | 60  |
| ttgactgccg gcgaaatgct gtttcgtgct ctccgcaagc actctcattt gcctcaagcc   | 120 |
| 40ttggtcgatg tggtcggcga tgaatctttg agctacaagg agttttttga ggcaaccgtc | 180 |

|              |            |            |            |             |            |      |
|--------------|------------|------------|------------|-------------|------------|------|
| ttgctggctc   | agtccctcca | caattgtggc | tacaagatga | acgacgtcgt  | tagtatctgt | 240  |
| gctgaaaaca   | ataccggtt  | cttcattcca | gtcatcgccg | catggtatat  | cggatgatc  | 300  |
| gtggctccag   | tcaacgagag | ctacattccc | gacgaactgt | gtaaagtcac  | gggtatctct | 360  |
| aagccacaga   | ttgtcttcac | cactaagaat | attctgaaca | aagtcctgga  | agtccaaagc | 420  |
| 5cgcaccaact  | ttattaagcg | tatcatcatc | ttggacactg | tggagaatat  | tcacggttgc | 480  |
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| ctcccaaagg   | gagtcacgca | gacccatcaa | aacattttgc | tgcgtctgat  | ccatgctctc | 660  |
| gatccacgct   | acggcactca | gctgattcct | ggtgtcaccc | tcttgggtcta | cttgccttcc | 720  |
| 10ttccatgctt | tcggctttca | tattactttg | ggttacttta | tggtcggtct  | ccgcgtgatt | 780  |
| atgttccgcc   | gttttgatca | ggaggctttc | ttgaaagcca | tccaagatta  | tgaagtccgc | 840  |
| agtgtcatca   | acgtgcctag | cgtgatcctg | tttttgtcta | agagcccact  | cgtggacaag | 900  |
| tacgacttgt   | cttcactgcg | tgaattgtgt | tgcggtgccg | ctccactggc  | taaggaggtc | 960  |
| gctgaagtgg   | ccgccaacg  | cttgaatcct | ccagggattc | gttgtggctt  | cggcctcacc | 1020 |
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| cgtgtcactc   | cactcatggc | tgctaagatc | gctgatcgcg | aaactggtaa  | ggctttgggc | 1140 |
| cctaaccaag   | tgggcgagct | gtgtatcaaa | ggccctatgg | tgagcaaggg  | ttatgtcaat | 1200 |
| aacgtcgaag   | ctaccaagga | ggccatcgac | gacgacggct | ggttgcatte  | tggtgatttt | 1260 |
| ggatattacg   | acgaagatga | gcatttttac | gtcgtggatc | gttacaagga  | gctgatcaaa | 1320 |
| 20tacaagggta | gccaggttgc | tccagctgag | ttggaggaga | ttctgttgaa  | aaatccatgc | 1380 |
| attcgcgatg   | tcgctgtggt | cggcattcct | gatctggagg | ccggcgaaact | gccttctgct | 1440 |
| ttcgttgtca   | agcagcctgg | taaagaaatt | accgccaaag | aagtgtatga  | ttacctggct | 1500 |
| gaacgtgtga   | gccatactaa | gtacttgctg | ggcggcgtgc | gttttgttga  | ctccatccct | 1560 |
| cgtaacgtaa   | caggcaaaat | taccgcgaag | gagctgttga | aacaattggt  | ggagaaggcc | 1620 |
| 25ggcgggt    |            |            |            |             |            | 1626 |

&lt;210&gt; 15

&lt;211&gt; 1626

&lt;212&gt; DNA

30&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Sequence of a synthetic luciferase

35&lt;400&gt; 15

|              |            |            |            |            |            |     |
|--------------|------------|------------|------------|------------|------------|-----|
| atgatgaagc   | gtgagaaaaa | tgtcatctat | ggccctgagc | ctctccatcc | tttggaggat | 60  |
| ttgactgccg   | gcgaaatgct | gtttcgtgct | ctccgcaagc | actcttattt | gcctcaagcc | 120 |
| ttggtcgatg   | tggtcggcga | tgaatctttg | agctacaagg | agttttttga | ggcaaccgtc | 180 |
| ttgctggctc   | agtccctcca | caattgtggc | tacaagatga | acgacgtcgt | tagtatctgt | 240 |
| 40gctgaaaaca | ataccggttt | cttcattcca | gtcatcgccg | catggtatat | cggatgatc  | 300 |

15

|              |             |            |            |            |            |            |      |
|--------------|-------------|------------|------------|------------|------------|------------|------|
| gtggctccag   | tcaacgagag  | ctacattccc | gacgaactgt | gtaaagtc   | cat        | gggtatctct | 360  |
| aagccacaga   | ttgtcttcac  | cactaagaat | attctgaaca | aagtcctgga | agtccaaagc |            | 420  |
| cgcaccaact   | ttattaagcg  | tatcatcatc | ttggacactg | tggagaatat | tcacgggtgc |            | 480  |
| gaatctttgc   | ctaatttcat  | ctctcgctat | tcagacggca | acatcgcaaa | ctttaaacca |            | 540  |
| 5ctccacttcg  | accctgtgga  | acaagttgca | gccattctgt | gtagcagcgg | tactactgga |            | 600  |
| ctcccaaagg   | gagtcatgca  | gacccatcaa | aacatttgcg | tgcgtctgat | ccatgctctc |            | 660  |
| gatccacgct   | acggcactca  | gctgattcct | gggtgcaccg | tcttggtcta | cttgcccttc |            | 720  |
| ttccatgctt   | tcggctttca  | tattactttg | ggttacttta | tggtcggtct | ccgcgtgatt |            | 780  |
| atgttccgcc   | gttttgatca  | ggaggctttc | ttgaaagcca | tccaagatta | tgaagtccgc |            | 840  |
| 10agtgtcatca | acgtgcctag  | cgtgatcctg | tttttgtcta | agagcccact | cgtggacaag |            | 900  |
| tacgacttgt   | cttccactgcg | tgaatttgtt | tgcggtgccg | ctccactggc | taaggaggtc |            | 960  |
| gctgaagtgg   | ccgccaaacg  | cttgaatctt | ccagggattc | gttgtggctt | cggcctcacc |            | 1020 |
| gaatctacca   | gcgctattat  | tcagtctctc | cgcgatgagt | ttaagagcgg | ctctttgggc |            | 1080 |
| cgtgtcactc   | cactcatggc  | tgctaagatc | gctgatcgcg | aaactggtaa | ggctttgggc |            | 1140 |
| 15ccgaaccaag | tgggcgagct  | gtgtatcaaa | ggccctatgg | tgagcaaggg | ttatgtcaat |            | 1200 |
| aacgttgaag   | ctaccaagga  | ggccatcgac | gacgacggct | ggttgcattc | tggtgatttt |            | 1260 |
| ggatattacg   | acgaagatga  | gcatttttac | gtcgtggatc | gttacaagga | gctgatcaaa |            | 1320 |
| tacaagggta   | gccaggttgc  | tccagctgag | ttggaggaga | ttctgttgaa | aaatccatgc |            | 1380 |
| attcgcgatg   | tcgctgtggt  | cggcattcct | gatctggagg | ccggcgaact | gccttctgct |            | 1440 |
| 20ttcgttgtca | agcagcctgg  | taaagaaatt | accgccaaag | aagtgtatga | ttacctggct |            | 1500 |
| gaacgtgtga   | gccatactaa  | gtacttgctg | ggcggcgtgc | gttttgttga | ctccatccct |            | 1560 |
| cgtaacgtaa   | caggcaaaat  | taccgcgaag | gagctgttga | aacaattggt | ggagaaggcc |            | 1620 |
| ggcgggt      |             |            |            |            |            |            | 1626 |

25&lt;210&gt; 16

&lt;211&gt; 1626

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

30&lt;220&gt;

&lt;223&gt; Sequence of a synthetic luciferase

&lt;400&gt; 16

|              |            |            |            |            |            |     |
|--------------|------------|------------|------------|------------|------------|-----|
| atgatgaagc   | gtgagaaaaa | tgtcatctat | ggccctgagc | ctctccatcc | tttggaggat | 60  |
| 35ttgactgccg | gcgaaatgct | gtttcgtgct | ctccgcaagc | actctcattt | gcctcaagcc | 120 |
| ttggctcgatg  | tggtcggcga | tgaatctttg | agctacaagg | agttttttga | ggcaaccgtc | 180 |
| ttgctggctc   | agtccctcca | caattgtggc | tacaagatga | acgacgtcgt | tagtatctgt | 240 |
| gctgaaaaca   | ataccggttt | cttcattcca | gtcatcgccg | catggtatat | cggtatgac  | 300 |
| gtggctccag   | tcaacgagag | ctacattccc | gacgaactgt | gtaaagtc   | cat        | 360 |
| 40aagccacaga | ttgtcttcac | cactaagaat | attctgaaca | aagtcctgga | agtccaaagc | 420 |

16

|   |      |
|---|------|
| cgcaccaact ttattaagcg tatcatcatc ttggacactg tggagaatat tcacgggttgc    | 480  |
| gaatcttttgc ctaatttcat ctctcgctat tcagacggca acatcgcaaa ctttaaacca    | 540  |
| ctccacttcg accctgtgga acaagttgca gccattctgt gtagcagcgg tactactgga     | 600  |
| ctcccaaagg gagtcatgca gacccatcaa aacattttgog tgcgtctgat ccatgctctc    | 660  |
| 5gatccacgct acggcactca gctgattcct ggtgtcacog tcttgggtcta cttgcctttc   | 720  |
| ttccatgctt tgggctttca tattactttg ggttacttta tggtcggtct ccgcgtgatt     | 780  |
| atgttcgcgc gttttgatca ggaggctttc ttgaaagcca tocaagatta tgaagtccgc     | 840  |
| agtgtcatca acgtgcctag cgtgatcctg tttttgtcta agagcccact cgtggacaag     | 900  |
| tacgacttgt cttcactgcg tgaatttgtt tgcggtgcog ctccactggc taaggaggtc     | 960  |
| 10gctgaagtgg ccgccaacg cttgaatctt ccagggatc gttgtggctt cggcctcacc     | 1020 |
| gaatctacca gcgctattat tcagtctctc cgcgatgagt ttaagagcgg ctctttgggc     | 1080 |
| cgtgtcactc cactcatggc tgctaagatc gctgatcgcg aaactggtaa ggctttgggc     | 1140 |
| ccgaaccaag tgggcgagct gtgtatcaaa ggccctatgg tgagcaaggg ttatgtcaat     | 1200 |
| aacgttgaag ctaccaagga ggccatcgac gacgacggct ggttgcattc tgggtgatttt    | 1260 |
| 15ggatattacg acgaagatga gcattttttac gtctgtggatc gttacaagga gctgatcaaa | 1320 |
| tacaagggtg gccaggttgc tccagctgag ttggaggaga ttctgttgaa aaatccatgc     | 1380 |
| attcgcgatg tcgctgtggt cggcattcct gatctggagg ccggcgaact gccttctgct     | 1440 |
| ttcgttgtca agcagcctgg taaagaaatt accgccaag aagtgtatga ttacctggct      | 1500 |
| gaacgtgtga gccatactaa gtacttgctt ggcggcgtgc gttttgttga ctccatcctt     | 1560 |
| 20cgtaacgtaa caggcaaat taccgcgaag gagctgttga aacaattgtt ggagaaggcc    | 1620 |
| ggcgggt   | 1626 |

&lt;210&gt; 17

&lt;211&gt; 1626

25&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Sequence of a synthetic luciferase

30

&lt;400&gt; 17

|  |     |
|--|-----|
| atgatgaagc gtgagaaaaa tgatcatctat ggccctgagc ctctccatcc tttggaggat   | 60  |
| ttgactgccg gcgaaatgct gtttcgtgct ctccgcaagc actctcattt gcctcaagcc    | 120 |
| ttggctgatg tggtcggcga tgaatctttg agctacaagg agttttttga ggcaaccgtc    | 180 |
| 35ttgctggctc agtccctcca caattgtggc tacaagatga acgacgtcgt tagtatctgt  | 240 |
| gctgaaaaca ataccggttt cttcattcca gtcacgccg catggtatat cggtatgac      | 300 |
| gtggctccag tcaacgagag ctacattccc gacgaactgt gttaaagtcac gggatctct    | 360 |
| aagccacaga ttgtcttcac cactaagaat attctgaaca aagtcttga agtccaaagc     | 420 |
| cgcaccaact ttattaagcg tatcatcatc ttggacactg tggagaatat tcacgggttgc   | 480 |
| 40gaatcttttgc ctaatttcat ctctcgctat tcagacggca acatcgcaaa ctttaaacca | 540 |

17

|  |      |
|--|------|
| ctccacttcg accctgtgga acaagttgca gccattctgt gtagcagcgg tactactgga    | 600  |
| ctcccaaagg gagtcatgca gacccatcaa aacatttgcg tgcgtctgat ccatgctctc    | 660  |
| gatccacgct acggcactca gctgattcct ggtgtcaccg tcttgggtcta cttgcctttc   | 720  |
| ttccatgctt tcggcttttca tattactttt gggttacttta tggtcggtct ccgcgtgatt  | 780  |
| 5atgttccgcc gttttgatca ggaggctttc ttgaaagcca tccaagatta tgaagtccgc   | 840  |
| agtgtcatca acgtgcctag cgtgatcctg tttttgtcta agagcccact cgtggacaag    | 900  |
| tacgacttgt cttcactgcg tgaatttgtt tgcgggtgcc ctccactggc taaggaggtc    | 960  |
| gctgaagtgg ccgccaacg cttgaatctt ccagggattc gttgtggctt cggcctcacc     | 1020 |
| gaatctacca gcgctattat tcagtctctc ggggatgagt ttaagagcgg ctctttgggc    | 1080 |
| 10cgtgtcactc cactcatggc tgctaagatc gctgatcgcg aaactggtaa ggctttgggc  | 1140 |
| ccgaaccaag tgggcgagct gtgtatcaaa ggccctatgg tgagcaaggg ttatgtcaat    | 1200 |
| aacgttgaag ctaccaagga ggccatcgac gacgacggct gggtgcattc tggatgtttt    | 1260 |
| ggatattacg acgaagatga gcattttttac gtctgtggatc gttacaagga gctgatcaaa  | 1320 |
| tacaagggta gccaggttgc tccagctgag ttggaggaga ttctgttgaa aaatccatgc    | 1380 |
| 15attcgcgatg tcgctgtggc cggcattcct gatctggagg ccggcgaaact gccttctgct | 1440 |
| ttcgttgtca agcagcctgg taaagaaatt accgccaag aagtgtatga ttacctggct     | 1500 |
| gaacgtgtga gccatactaa gtacttgcgt ggcggcgtgc gttttgttga ctccatccct    | 1560 |
| cgtaacgtaa caggcaaaat taccgcgaag gagctgttga aacaattgtt ggagaaggcc    | 1620 |
| ggcggt   | 1626 |

20

&lt;210&gt; 18

&lt;211&gt; 1626

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

25

&lt;220&gt;

&lt;223&gt; Sequence of a synthetic luciferase

&lt;400&gt; 18

|  |     |
|--|-----|
| 30atgataaagc gtgagaaaaa tgatcatctat ggccctgagc ctctccatcc tttggaggat | 60  |
| ttgactgccg gcgaaatgct gtttcgtgct ctccgcaagc actctcattt gcctcaagcc    | 120 |
| ttggctgatg tggtcggcga tgaatctttg agctacaagg agttttttga ggcaaccgtc    | 180 |
| ttgctggctc agtccctoca caattgtggc tacaagatga acgacgtcgt tagtatctgt    | 240 |
| gctgaaaaca ataccggttt cttcattcca gtcatcgccg catggatatat cggtatgac    | 300 |
| 35gtggctccag tcaacgagag ctacattccc gacgaactgt gttaaagtcac gggatatctc | 360 |
| aagccacaga ttgtcttcac cactaagaat attctgaaca aagtcctgga agtccaaagc    | 420 |
| cgcaccaact ttattaagcg tatcatcatc ttggacactg tggagaatat tcacggttgc    | 480 |
| gaatctttgc ctaatttcat ctctcgctat tcagacggca acatcgcaaa ctttaaacca    | 540 |
| ctccacttcg accctgtgga acaagttgca gccattctgt gtagcagcgg tactactgga    | 600 |
| 40ctcccaaagg gagtcatgca gacccatcaa aacatttgcg tgcgtctgat ccatgctctc  | 660 |

|              |             |             |             |            |             |      |
|--------------|-------------|-------------|-------------|------------|-------------|------|
| gatccacgct   | acggcactca  | gctgattcct  | ggtgtcaccg  | tcttggtcta | cttgcccttc  | 720  |
| ttccatgctt   | tcggctttca  | tattactttg  | ggttacttta  | tggtcgggtc | ccgcgtgatt  | 780  |
| atggtccgcc   | gttttgatca  | ggaggctttc  | ttgaaagcca  | tccaagatta | tgaagtccgc  | 840  |
| agtgtcatca   | acgtgcctag  | cgtgatcctg  | tttttgtcta  | agagcccact | cgtggacaag  | 900  |
| 5tacgacttgt  | cttcaactgcg | tgaatttgtt  | tgcggtgccg  | ctccactggc | taaggagggtc | 960  |
| gctgaagtgg   | ccgccaaaacg | cttgaatctt  | ccagggatcc  | gttgtggctt | cggcctcacc  | 1020 |
| gaatctacca   | gtgcgattat  | ccagactctc  | ggggatgagt  | ttaagagcgg | ctctttgggc  | 1080 |
| cgtgtcactc   | cactcatggc  | tgctaagatc  | gctgatcgcg  | aaactggtaa | ggctttgggc  | 1140 |
| ccgaaccaag   | tgggcgagct  | gtgtatcaaa  | ggccctatgg  | tgagcaaggg | ttatgtcaat  | 1200 |
| 10aacgttgaag | ctaccaagga  | ggccatcgac  | gacgacggct  | ggttgcatcc | tggtgatttt  | 1260 |
| ggatattacg   | acgaagatga  | gcattttttac | gtcgtggatc  | gttacaagga | gctgatcaaa  | 1320 |
| tacaagggta   | gccagggttg  | tccagctgag  | ttggaggaga  | ttctgttgaa | aaatccatgc  | 1380 |
| attcgcgatg   | tcgctgtggt  | cggcattcct  | gatctggagg  | ccggcgaact | gccttctgct  | 1440 |
| ttcgttgtca   | agcagcctgg  | tacagaaatt  | accgccaaaag | aagtgtatga | ttacctggct  | 1500 |
| 15gaacgtgtga | gccatactaa  | gtacttgctg  | ggcggcgtgc  | gttttgttga | ctccatccct  | 1560 |
| cgtaacgtaa   | caggcaaaat  | taccgcgaag  | gagctgttga  | aacaattgtt | ggtgaaggcc  | 1620 |
| ggcgggt      |             |             |             |            |             | 1626 |

&lt;210&gt; 19

20&lt;211&gt; 933

&lt;212&gt; DNA

&lt;213&gt; Renilla reniformis

&lt;400&gt; 19

|              |            |             |            |             |             |     |
|--------------|------------|-------------|------------|-------------|-------------|-----|
| 25atgacttcga | aagtttatga | tccagaacaa  | aggaaacgga | tgataactgg  | tcgcagtggt  | 60  |
| tgggccagat   | gtaaacaat  | gaatgttctt  | gattcattta | ttaattatta  | tgattcagaa  | 120 |
| aaacatgcag   | aaaatgctgt | tattttttta  | catggtaacg | cggcctcttc  | ttattttatgg | 180 |
| cgacatgttg   | tgccacatat | tgagccagta  | gcgcggtgta | ttataccaga  | tcttattgggt | 240 |
| atgggcaaat   | caggcaaatc | tggtaatgggt | tcttataggt | tacttgatca  | ttacaaatat  | 300 |
| 30cttactgcat | ggtttgaact | tcttaattta  | ccaaagaaga | tcattttttgt | cggccatgat  | 360 |
| tggggtgctt   | gtttggcatt | tcattatagc  | tatgagcatc | aagataagat  | caaagcaata  | 420 |
| gttcacgctg   | aaagtgtagt | agatgtgatt  | gaatcatggg | atgaatggcc  | tgatattgaa  | 480 |
| gaagatattg   | cgttgatcaa | atctgaagaa  | ggagaaaaaa | tggttttgga  | gaataacttc  | 540 |
| ttcgtggaaa   | ccatgttgcc | atcaaaaatc  | atgagaaagt | tagaaccaga  | agaatttgca  | 600 |
| 35gcatatcttg | aaccattcaa | agagaaaggt  | gaagttcgct | gtccaacatt  | atcatggcct  | 660 |
| cgtgaaatcc   | cgtagtaaaa | agggtgtaaa  | cctgacgttg | tacaaattgt  | taggaattat  | 720 |
| aatgcttata   | tacgtgcaag | tgatgattta  | ccaaaaatgt | ttattgaatc  | ggatccagga  | 780 |
| ttcttttcca   | atgctattgt | tgaaggcgcc  | aagaagtttc | ctaatactga  | atttgtcaaa  | 840 |
| gtaaaagggtc  | ttcatttttc | gcaagaagat  | gcacctgatg | aaatgggaaa  | atatatcaaa  | 900 |
| 40tcgttcgttg | agcgagttct | caaaaatgaa  | caa        |             |             | 933 |



&lt;210&gt; 20

&lt;211&gt; 933

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

5

&lt;220&gt;

&lt;223&gt; Sequence of a synthetic luciferase

&lt;400&gt; 20

|  |     |
|--|-----|
| 10atggccttcca aggtgtacga ccccgagcag cgcaagcgca tgatcacccg ccctcagtgg | 60  |
| tgggcccgct gcaagcagat gaacgtgctg gactccttca tcaactacta cgacagcgag    | 120 |
| aagcacgccg agaacgccgt gatcttcctg cacggcaacg ccgcctccag ctacctgtgg    | 180 |
| aggcacgtgg tgcctcacat cgagcccgtg gcccgtgca tcatccctga cctgatcggc     | 240 |
| atgggcaagt ccggcaagag cggcaacggc tcctaccgcc tgctggacca ctacaagtac    | 300 |
| 15ctgaccgcct ggttcgagct gctgaacctg cccaagaaga tcatcttcgt gggccacgac  | 360 |
| tggggagcct gcctggcctt ccactactcc tacgagcaac aggacaagat caaggccatc    | 420 |
| gtgcacgccg agagcgtggt ggacgtgacg gactcctggg acgagtggcc tgacatcgag    | 480 |
| gaggacatcg ccctgatcaa gagcgaggag ggcgagaaga tggtgctgga gaacaacttc    | 540 |
| ttcgtggaga ccatgctgcc cagcaagatc atgcgcaagc tggagcctga ggagttcgcc    | 600 |
| 20gcctacctgg agcccttcaa ggagaagggc gaggtgcgcc gccctaccct gtcctggccc  | 660 |
| cgcgagatcc ctctggtgaa gggcggaag cccgacgtgg tgcagatcgt gcgcaactac     | 720 |
| aacgcctacc tgcgcgccag cgacgacctg cctaagatgt tcatcgagtc cgaccctggc    | 780 |
| ttcttctcca acgccatcgt cgagggagcc aagaagttcc ccaacaccga gttcgtgaag    | 840 |
| gtgaagggcc tgcacttctc ccaggaggac gcccctgacg agatgggcaa gtacatcaag    | 900 |
| 25agcttcgtgg agcgcgtgct gaagaacgag cag                               | 933 |

&lt;210&gt; 21

&lt;211&gt; 933

&lt;212&gt; DNA

30&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Sequence of a synthetic luciferase

35&lt;400&gt; 21

|   |     |
|---|-----|
| atggccttcca aggtgtacga ccccgagcaa cgcaaacgca tgatcactgg gcctcagtgg  | 60  |
| tgggctcgct gcaagcaaat gaacgtgctg gactccttca tcaactacta tgattccgag   | 120 |
| aagcacgccg agaacgccgt gatttttctg catggtaacg ctgcctccag ctacctgtgg   | 180 |
| aggcacgtcg tgcctcacat cgagcccgtg gctcgctgca tcatccctga tctgatcgga   | 240 |
| 40atgggtaagt ccggcaagag cgggaatggc tcatatcgcc tcctggatca ctacaagtac | 300 |

20

```

ctcaccgott ggttcgagct gctgaacctt ccaaagaaaa tcatctttgt gggccacgac      360
tggtgggctt gtctggcctt tctactactcc tacgagcacc aagacaagat caaggccatc      420
gtccatgctg agagtgtcgt ggacgtgata ggtcctggg acgagtggcc tgacatcgag      480
gaggatatcg ccctgatcaa gagcgaagag ggcgagaaaa tgggtgcttga gaataacttc      540
5ttcgtcgaga ccatgctccc aagcaagatc atgcggaaac tggagcctga ggagttcgct      600
gcctacctgg agcccttcaa ggagaagggc gaggttagac ggcctaccct ctctggcct      660
cgcgagatcc ctctcgtaa gggaggcaag cccgacgtcg tccagattgt ccgcaactac      720
aacgcctacc ttcgggccag cgacgatctg cctaagatgt tcatcgagtc cgaccctggg      780
ttcttttcca acgctattgt cgaggagct aagaagttcc ctaacaccga gttcgtgaag      840
10gtgaagggcc tccacttcag ccaggaggac gctccagatg aaatgggtaa gtacatcaag      900
agcttcgtgg agcgcgtgct gaagaacgag cag      933

```

&lt;210&gt; 22

&lt;211&gt; 933

15&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Sequence of a synthetic luciferase

20

&lt;400&gt; 22

```

atggcttcca aggtgtacga ccccgagcaa cgcaaacgca tgatcactgg gcctcagtgg      60
tggttcgctt gcaagcaaatt gaacgtgctg gactccttca tcaactacta tgattccgag      120
aagcacgccg agaacgccgt gatttttctg catggtaacg ctgcctccag ctacctgtgg      180
25aggcacgtcg tgcctcacat cgagcccgtg gctagatgca tcatccctga tctgatcgga      240
atgggtaagt cgggcaagag cgggaatggc tcatatcgcc tcctggatca ctacaagtac      300
ctcaccgctt ggttcgagct gctgaacctt ccaaagaaaa tcatctttgt gggccacgac      360
tggtgggctt gtctggcctt tctactactcc tacgagcacc aagacaagat caaggccatc      420
gtccatgctg agagtgtcgt ggacgtgata ggtcctggg acgagtggcc tgacatcgag      480
30gaggatatcg ccctgatcaa gagcgaagag ggcgagaaaa tgggtgcttga gaataacttc      540
ttcgtcgaga ccatgctccc aagcaagatc atgcggaaac tggagcctga ggagttcgct      600
gcctacctgg agccattcaa ggagaagggc gaggttagac ggcctaccct ctctggcct      660
cgcgagatcc ctctcgtaa gggaggcaag cccgacgtcg tccagattgt ccgcaactac      720
aacgcctacc ttcgggccag cgacgatctg cctaagatgt tcatcgagtc cgaccctggg      780
35ttcttttcca acgctattgt cgaggagct aagaagttcc ctaacaccga gttcgtgaag      840
gtgaagggcc tccacttcag ccaggaggac gctccagatg aaatgggtaa gtacatcaag      900
agcttcgtgg agcgcgtgct gaagaacgag cag      933

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&lt;210&gt; 23

40&lt;211&gt; 543

21

&lt;212&gt; PRT

&lt;213&gt; Pyrophorus plagiophthalmus

&lt;400&gt; 23

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Met Met Lys Arg Glu Lys Asn Val Ile Tyr Gly Pro Glu Pro Leu His
 1           5           10           15
Pro Leu Glu Asp Leu Thr Ala Gly Glu Met Leu Phe Arg Ala Leu Arg
          20           25           30
Lys His Ser His Leu Pro Gln Ala Leu Val Asp Val Phe Gly Asp Glu
10      35           40           45
Ser Leu Ser Tyr Lys Glu Phe Phe Glu Ala Thr Cys Leu Leu Ala Gln
      50           55           60
Ser Leu His Asn Cys Gly Tyr Lys Met Asn Asp Val Val Ser Ile Cys
65           70           75           80
15Ala Glu Asn Asn Lys Arg Phe Phe Ile Pro Ile Ile Ala Ala Trp Tyr
          85           90           95
Ile Gly Met Ile Val Ala Pro Val Asn Glu Ser Tyr Ile Pro Asp Glu
          100          105          110
Leu Cys Lys Val Met Gly Ile Ser Lys Pro Gln Ile Val Phe Cys Thr
20      115          120          125
Lys Asn Ile Leu Asn Lys Val Leu Glu Val Gln Ser Arg Thr Asn Phe
      130          135          140
Ile Lys Arg Ile Ile Ile Leu Asp Thr Val Glu Asn Ile His Gly Cys
145          150          155          160
25Glu Ser Leu Pro Asn Phe Ile Ser Arg Tyr Ser Asp Gly Asn Ile Ala
          165          170          175
Asn Phe Lys Pro Leu His Tyr Asp Pro Val Glu Gln Val Ala Ala Ile
          180          185          190
Leu Cys Ser Ser Gly Thr Thr Gly Leu Pro Lys Gly Val Met Gln Thr
30      195          200          205
His Gln Asn Ile Cys Val Arg Leu Ile His Ala Leu Asp Pro Arg Ala
      210          215          220
Gly Thr Gln Leu Ile Pro Gly Val Thr Val Leu Val Tyr Leu Pro Phe
225          230          235          240
35Phe His Ala Phe Gly Phe Ser Ile Asn Leu Gly Tyr Phe Met Val Gly
          245          250          255
Leu Arg Val Ile Met Leu Arg Arg Phe Asp Gln Glu Ala Phe Leu Lys
          260          265          270
Ala Ile Gln Asp Tyr Glu Val Arg Ser Val Ile Asn Val Pro Ala Ile
40      275          280          285

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22

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Ile Leu Phe Leu Ser Lys Ser Pro Leu Val Asp Lys Tyr Asp Leu Ser
  290                      295                      300
Ser Leu Arg Glu Leu Cys Cys Gly Ala Ala Pro Leu Ala Lys Glu Val
305                      310                      315                      320
15Ala Glu Val Ala Val Lys Arg Leu Asn Leu Pro Gly Ile Arg Cys Gly
                      325                      330                      335
Phe Gly Leu Thr Glu Ser Thr Ser Ala Asn Ile His Ser Leu Gly Asp
                      340                      345                      350
Glu Phe Lys Ser Gly Ser Leu Gly Arg Val Thr Pro Leu Met Ala Ala
10      355                      360                      365
Lys Ile Ala Asp Arg Glu Thr Gly Lys Ala Leu Gly Pro Asn Gln Val
  370                      375                      380
Gly Glu Leu Cys Val Lys Gly Pro Met Val Ser Lys Gly Tyr Val Asn
385                      390                      395                      400
15Asn Val Glu Ala Thr Lys Glu Ala Ile Asp Asp Asp Gly Trp Leu His
                      405                      410                      415
Ser Gly Asp Phe Gly Tyr Tyr Asp Glu Asp Glu His Phe Tyr Val Val
                      420                      425                      430
Asp Arg Tyr Lys Glu Leu Ile Lys Tyr Lys Gly Ser Gln Val Ala Pro
20      435                      440                      445
Ala Glu Leu Glu Glu Ile Leu Leu Lys Asn Pro Cys Ile Arg Asp Val
  450                      455                      460
Ala Val Val Gly Ile Pro Asp Leu Glu Ala Gly Glu Leu Pro Ser Ala
465                      470                      475                      480
25Phe Val Val Lys Gln Pro Gly Lys Glu Ile Thr Ala Lys Glu Val Tyr
                      485                      490                      495
Asp Tyr Leu Ala Glu Arg Val Ser His Thr Lys Tyr Leu Arg Gly Gly
                      500                      505                      510
Val Arg Phe Val Asp Ser Ile Pro Arg Asn Val Thr Gly Lys Ile Thr
30      515                      520                      525
Arg Lys Glu Leu Leu Lys Gln Leu Leu Glu Lys Ser Ser Lys Leu
  530                      535                      540

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&lt;210&gt; 24

35&lt;211&gt; 542

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

40&lt;223&gt; Sequence of clone YG#81-6G01

&lt;400&gt; 24

|       |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met   | Met | Lys | Arg | Glu | Lys | Asn | Val | Ile | Tyr | Gly | Pro | Glu | Pro | Leu | His |
| 1     |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |
| Pro   | Leu | Glu | Asp | Leu | Thr | Ala | Gly | Glu | Met | Leu | Phe | Arg | Ala | Leu | Arg |
| 5     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |
| Lys   | His | Ser | His | Leu | Pro | Gln | Ala | Leu | Val | Asp | Val | Val | Gly | Asp | Glu |
|       |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |
| Ser   | Leu | Ser | Tyr | Lys | Glu | Phe | Phe | Glu | Ala | Thr | Val | Leu | Leu | Ala | Gln |
|       | 50  |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |
| 10Ser | Leu | His | Asn | Cys | Gly | Tyr | Lys | Met | Asn | Asp | Val | Val | Ser | Ile | Cys |
| 65    |     |     |     | 70  |     |     |     |     |     | 75  |     |     |     | 80  |     |
| Ala   | Glu | Asn | Asn | Thr | Arg | Phe | Phe | Ile | Pro | Val | Ile | Ala | Ala | Trp | Tyr |
|       |     |     | 85  |     |     |     |     | 90  |     |     |     |     | 95  |     |     |
| Ile   | Gly | Met | Ile | Val | Ala | Pro | Val | Asn | Glu | Ser | Tyr | Ile | Pro | Asp | Glu |
| 15    |     |     | 100 |     |     |     |     | 105 |     |     |     |     | 110 |     |     |
| Leu   | Cys | Lys | Val | Met | Gly | Ile | Ser | Lys | Pro | Gln | Ile | Val | Phe | Thr | Thr |
|       |     | 115 |     |     |     |     | 120 |     |     |     |     | 125 |     |     |     |
| Lys   | Asn | Ile | Leu | Asn | Lys | Val | Leu | Glu | Val | Gln | Ser | Arg | Thr | Asn | Phe |
|       | 130 |     |     |     |     | 135 |     |     |     |     | 140 |     |     |     |     |
| 20Ile | Lys | Arg | Ile | Ile | Ile | Leu | Asp | Thr | Val | Glu | Asn | Ile | His | Gly | Cys |
| 145   |     |     |     | 150 |     |     |     |     |     | 155 |     |     |     | 160 |     |
| Glu   | Ser | Leu | Pro | Asn | Phe | Ile | Ser | Arg | Tyr | Ser | Asp | Gly | Asn | Ile | Ala |
|       |     |     | 165 |     |     |     |     | 170 |     |     |     |     | 175 |     |     |
| Asn   | Phe | Lys | Pro | Leu | His | Phe | Asp | Pro | Val | Glu | Gln | Val | Ala | Ala | Ile |
| 25    |     |     | 180 |     |     |     |     | 185 |     |     |     |     | 190 |     |     |
| Leu   | Cys | Ser | Ser | Gly | Thr | Thr | Gly | Leu | Pro | Lys | Gly | Val | Met | Gln | Thr |
|       |     | 195 |     |     |     |     | 200 |     |     |     |     | 205 |     |     |     |
| His   | Gln | Asn | Ile | Cys | Val | Arg | Leu | Ile | His | Ala | Leu | Asp | Pro | Arg | Ala |
|       | 210 |     |     |     |     | 215 |     |     |     |     |     | 220 |     |     |     |
| 30Gly | Thr | Gln | Leu | Ile | Pro | Gly | Val | Thr | Val | Leu | Val | Tyr | Leu | Pro | Phe |
| 225   |     |     |     | 230 |     |     |     |     |     | 235 |     |     |     | 240 |     |
| Phe   | His | Ala | Phe | Gly | Phe | Ser | Ile | Thr | Leu | Gly | Tyr | Phe | Met | Val | Gly |
|       |     |     | 245 |     |     |     |     | 250 |     |     |     |     | 255 |     |     |
| Leu   | Arg | Val | Ile | Met | Phe | Arg | Arg | Phe | Asp | Gln | Glu | Ala | Phe | Leu | Lys |
| 35    |     |     | 260 |     |     |     |     | 265 |     |     |     |     | 270 |     |     |
| Ala   | Ile | Gln | Asp | Tyr | Glu | Val | Arg | Ser | Val | Ile | Asn | Val | Pro | Ser | Val |
|       |     | 275 |     |     |     |     | 280 |     |     |     |     | 285 |     |     |     |
| Ile   | Leu | Phe | Leu | Ser | Lys | Ser | Pro | Leu | Val | Asp | Lys | Tyr | Asp | Leu | Ser |
|       | 290 |     |     |     |     | 295 |     |     |     |     |     | 300 |     |     |     |
| 40Ser | Leu | Arg | Glu | Leu | Cys | Cys | Gly | Ala | Ala | Pro | Leu | Ala | Lys | Glu | Val |

24

305                      310                      315                      320  
 Ala Glu Val Ala Ala Lys Arg Leu Asn Leu Pro Gly Ile Arg Cys Gly  
                          325                      330                      335  
 Phe Gly Leu Thr Glu Ser Thr Ser Ala Asn Ile His Ser Leu Arg Asp  
 5                      340                      345                      350  
 Glu Phe Lys Ser Gly Ser Leu Gly Arg Val Thr Pro Leu Met Ala Ala  
                          355                      360                      365  
 Lys Ile Ala Asp Arg Glu Thr Gly Lys Ala Leu Gly Pro Asn Gln Val  
                          370                      375                      380  
 10Gly Glu Leu Cys Ile Lys Gly Pro Met Val Ser Lys Gly Tyr Val Asn  
                          385                      390                      395                      400  
 Asn Val Glu Ala Thr Lys Glu Ala Ile Asp Asp Asp Gly Trp Leu His  
                          405                      410                      415  
 Ser Gly Asp Phe Gly Tyr Tyr Asp Glu Asp Glu His Phe Tyr Val Val  
 15                      420                      425                      430  
 Asp Arg Tyr Lys Glu Leu Ile Lys Tyr Lys Gly Ser Gln Val Ala Pro  
                          435                      440                      445  
 Ala Glu Leu Glu Glu Ile Leu Leu Lys Asn Pro Cys Ile Arg Asp Val  
                          450                      455                      460  
 20Ala Val Val Gly Ile Pro Asp Leu Glu Ala Gly Glu Leu Pro Ser Ala  
                          465                      470                      475                      480  
 Phe Val Val Lys Gln Pro Gly Lys Glu Ile Thr Ala Lys Glu Val Tyr  
                          485                      490                      495  
 Asp Tyr Leu Ala Glu Arg Val Ser His Thr Lys Tyr Leu Arg Gly Gly  
 25                      500                      505                      510  
 Val Arg Phe Val Asp Ser Ile Pro Arg Asn Val Thr Gly Lys Ile Thr  
                          515                      520                      525  
 Arg Lys Glu Leu Leu Lys Gln Leu Leu Glu Lys Ala Gly Gly  
                          530                      535                      540

30

&lt;210&gt; 25

&lt;211&gt; 542

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

35

&lt;220&gt;

&lt;223&gt; Sequence of a synthetic luciferase

&lt;400&gt; 25

40Met Met Lys Arg Glu Lys Asn Val Ile Tyr Gly Pro Glu Pro Leu His

| 25  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |  |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|--|
| 1   | 5   |     |     |     | 10  |     |     |     | 15  |     |     |     |     |     |     |     |  |  |
| Pro | Leu | Glu | Asp | Leu | Thr | Ala | Gly | Glu | Met | Leu | Phe | Arg | Ala | Leu | Arg |     |  |  |
| 20  |     |     |     | 25  |     |     |     | 30  |     |     |     |     |     |     |     |     |  |  |
| Lys | His | Ser | His | Leu | Pro | Gln | Ala | Leu | Val | Asp | Val | Val | Gly | Asp | Glu |     |  |  |
| 5   | 35  |     |     | 40  |     |     | 45  |     |     |     |     |     |     |     |     |     |  |  |
| Ser | Leu | Ser | Tyr | Lys | Glu | Phe | Phe | Glu | Ala | Thr | Val | Leu | Leu | Ala | Gln |     |  |  |
| 50  |     |     | 55  |     |     | 60  |     |     |     |     |     |     |     |     |     |     |  |  |
| Ser | Leu | His | Asn | Cys | Gly | Tyr | Lys | Met | Asn | Asp | Val | Val | Ser | Ile | Cys |     |  |  |
| 65  | 70  |     |     |     | 75  |     |     |     | 80  |     |     |     |     |     |     |     |  |  |
| 10  | Ala | Glu | Asn | Asn | Thr | Arg | Phe | Phe | Ile | Pro | Val | Ile | Ala | Ala | Trp | Tyr |  |  |
| 85  |     |     |     | 90  |     |     |     | 95  |     |     |     |     |     |     |     |     |  |  |
| Ile | Gly | Met | Ile | Val | Ala | Pro | Val | Asn | Glu | Ser | Tyr | Ile | Pro | Asp | Glu |     |  |  |
| 100 |     |     | 105 |     |     | 110 |     |     |     |     |     |     |     |     |     |     |  |  |
| Leu | Cys | Lys | Val | Met | Gly | Ile | Ser | Lys | Pro | Gln | Ile | Val | Phe | Thr | Thr |     |  |  |
| 15  | 115 |     |     | 120 |     |     | 125 |     |     |     |     |     |     |     |     |     |  |  |
| Lys | Asn | Ile | Leu | Asn | Lys | Val | Leu | Glu | Val | Gln | Ser | Arg | Thr | Asn | Phe |     |  |  |
| 130 |     |     | 135 |     |     | 140 |     |     |     |     |     |     |     |     |     |     |  |  |
| Ile | Lys | Arg | Ile | Ile | Ile | Leu | Asp | Thr | Val | Glu | Asn | Ile | His | Gly | Cys |     |  |  |
| 145 | 150 |     |     |     | 155 |     |     |     | 160 |     |     |     |     |     |     |     |  |  |
| 20  | Glu | Ser | Leu | Pro | Asn | Phe | Ile | Ser | Arg | Tyr | Ser | Asp | Gly | Asn | Ile | Ala |  |  |
| 165 |     |     |     | 170 |     |     |     | 175 |     |     |     |     |     |     |     |     |  |  |
| Asn | Phe | Lys | Pro | Leu | His | Phe | Asp | Pro | Val | Glu | Gln | Val | Ala | Ala | Ile |     |  |  |
| 180 |     |     | 185 |     |     | 190 |     |     |     |     |     |     |     |     |     |     |  |  |
| Leu | Cys | Ser | Ser | Gly | Thr | Thr | Gly | Leu | Pro | Lys | Gly | Val | Met | Gln | Thr |     |  |  |
| 25  | 195 |     |     | 200 |     |     | 205 |     |     |     |     |     |     |     |     |     |  |  |
| His | Gln | Asn | Ile | Cys | Val | Arg | Leu | Ile | His | Ala | Leu | Asp | Pro | Arg | Val |     |  |  |
| 210 |     |     | 215 |     |     | 220 |     |     |     |     |     |     |     |     |     |     |  |  |
| Gly | Thr | Gln | Leu | Ile | Pro | Gly | Val | Thr | Val | Leu | Val | Tyr | Leu | Pro | Phe |     |  |  |
| 225 | 230 |     |     |     | 235 |     |     |     | 240 |     |     |     |     |     |     |     |  |  |
| 30  | Phe | His | Ala | Phe | Gly | Phe | Ser | Ile | Thr | Leu | Gly | Tyr | Phe | Met | Val | Gly |  |  |
| 245 |     |     |     | 250 |     |     |     | 255 |     |     |     |     |     |     |     |     |  |  |
| Leu | Arg | Val | Ile | Met | Phe | Arg | Arg | Phe | Asp | Gln | Glu | Ala | Phe | Leu | Lys |     |  |  |
| 260 |     |     | 265 |     |     | 270 |     |     |     |     |     |     |     |     |     |     |  |  |
| Ala | Ile | Gln | Asp | Tyr | Glu | Val | Arg | Ser | Val | Ile | Asn | Val | Pro | Ser | Val |     |  |  |
| 35  | 275 |     |     | 280 |     |     | 285 |     |     |     |     |     |     |     |     |     |  |  |
| Ile | Leu | Phe | Leu | Ser | Lys | Ser | Pro | Leu | Val | Asp | Lys | Tyr | Asp | Leu | Ser |     |  |  |
| 290 |     |     | 295 |     |     | 300 |     |     |     |     |     |     |     |     |     |     |  |  |
| Ser | Leu | Arg | Glu | Leu | Cys | Cys | Gly | Ala | Ala | Pro | Leu | Ala | Lys | Glu | Val |     |  |  |
| 305 | 310 |     |     |     | 315 |     |     |     | 320 |     |     |     |     |     |     |     |  |  |
| 40  | Ala | Glu | Val | Ala | Ala | Lys | Arg | Leu | Asn | Leu | Pro | Gly | Ile | Arg | Cys | Gly |  |  |

26

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          325          330          335
Phe Gly Leu Thr Glu Ser Thr Ser Ala Asn Ile His Ser Leu Arg Asp
          340          345          350
-----
Glu Phe Lys Ser Gly Ser Leu Gly Arg Val Thr Pro Leu Met Ala Ala
5          355          360          365
Lys Ile Ala Asp Arg Glu Thr Gly Lys Ala Leu Gly Pro Asn Gln Val
          370          375          380
Gly Glu Leu Cys Ile Lys Gly Pro Met Val Ser Lys Gly Tyr Val Asn
385          390          395          400
10Asn Val Glu Ala Thr Lys Glu Ala Ile Asp Asp Asp Gly Trp Leu His
          405          410          415
Ser Gly Asp Phe Gly Tyr Tyr Asp Glu Asp Glu His Phe Tyr Val Val
          420          425          430
Asp Arg Tyr Lys Glu Leu Ile Lys Tyr Lys Gly Ser Gln Val Ala Pro
15          435          440          445
Ala Glu Leu Glu Glu Ile Leu Leu Lys Asn Pro Cys Ile Arg Asp Val
          450          455          460
Ala Val Val Gly Ile Pro Asp Leu Glu Ala Gly Glu Leu Pro Ser Ala
465          470          475          480
20Phe Val Val Lys Gln Pro Gly Lys Glu Ile Thr Ala Lys Glu Val Tyr
          485          490          495
Asp Tyr Leu Ala Glu Arg Val Ser His Thr Lys Tyr Leu Arg Gly Gly
          500          505          510
Val Arg Phe Val Asp Ser Ile Pro Arg Asn Val Thr Gly Lys Ile Thr
25          515          520          525
Arg Lys Glu Leu Leu Lys Gln Leu Leu Glu Lys Ala Gly Gly
          530          535          540

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&lt;210&gt; 26

30&lt;211&gt; 542

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

35&lt;223&gt; Sequence of a synthetic luciferase

&lt;400&gt; 26

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Met Met Lys Arg Glu Lys Asn Val Ile Tyr Gly Pro Glu Pro Leu His
1          5          10          15
40Pro Leu Glu Asp Leu Thr Ala Gly Glu Met Leu Phe Arg Ala Leu Arg

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27

20 25 30  
 Lys His Ser His Leu Pro Gln Ala Leu Val Asp Val Val Gly Asp Glu  
 35 40 45  
 Ser Leu Ser Tyr Lys Glu Phe Phe Glu Ala Thr Val Leu Leu Ala Gln  
 5 50 55 60  
 Ser Leu His Asn Cys Gly Tyr Lys Met Asn Asp Val Val Ser Ile Cys  
 65 70 75 80  
 Ala Glu Asn Asn Thr Arg Phe Phe Ile Pro Val Ile Ala Ala Trp Tyr  
 85 90 95  
 10 Ile Gly Met Ile Val Ala Pro Val Asn Glu Ser Tyr Ile Pro Asp Glu  
 100 105 110  
 Leu Cys Lys Val Met Gly Ile Ser Lys Pro Gln Ile Val Phe Thr Thr  
 115 120 125  
 Lys Asn Ile Leu Asn Lys Val Leu Glu Val Gln Ser Arg Thr Asn Phe  
 15 130 135 140  
 Ile Lys Arg Ile Ile Ile Leu Asp Thr Val Glu Asn Ile His Gly Cys  
 145 150 155 160  
 Glu Ser Leu Pro Asn Phe Ile Ser Arg Tyr Ser Asp Gly Asn Ile Ala  
 165 170 175  
 20 Asn Phe Lys Pro Leu His Phe Asp Pro Val Glu Gln Val Ala Ala Ile  
 180 185 190  
 Leu Cys Ser Ser Gly Thr Thr Gly Leu Pro Lys Gly Val Met Gln Thr  
 195 200 205  
 His Gln Asn Ile Cys Val Arg Leu Ile His Ala Leu Asp Pro Arg Val  
 25 210 215 220  
 Gly Thr Gln Leu Ile Pro Gly Val Thr Val Leu Val Tyr Leu Pro Phe  
 225 230 235 240  
 Phe His Ala Phe Gly Phe Ser Ile Thr Leu Gly Tyr Phe Met Val Gly  
 245 250 255  
 30 Leu Arg Val Ile Met Phe Arg Arg Phe Asp Gln Glu Ala Phe Leu Lys  
 260 265 270  
 Ala Ile Gln Asp Tyr Glu Val Arg Ser Val Ile Asn Val Pro Ser Val  
 275 280 285  
 Ile Leu Phe Leu Ser Lys Ser Pro Leu Val Asp Lys Tyr Asp Leu Ser  
 35 290 295 300  
 Ser Leu Arg Glu Leu Cys Cys Gly Ala Ala Pro Leu Ala Lys Glu Val  
 305 310 315 320  
 Ala Glu Val Ala Ala Lys Arg Leu Asn Leu Pro Gly Ile Arg Cys Gly  
 325 330 335  
 40 Phe Gly Leu Thr Glu Ser Thr Ser Ala Asn Ile His Ser Leu Arg Asp

28

|    |   |     |     |     |     |
|----|---|-----|-----|-----|-----|
|    | 340   |     | 345 |     | 350 |
|    | Glu Phe Lys Ser Gly Ser Leu Gly Arg Val Thr Pro Leu Met Ala Ala |     |     |     |     |
|    | 355   |     | 360 |     | 365 |
|    | Lys Ile Ala Asp Arg Glu Thr Gly Lys Ala Leu Gly Pro Asn Gln Val |     |     |     |     |
| 5  | 370   |     | 375 |     | 380 |
|    | Gly Glu Leu Cys Ile Lys Gly Pro Met Val Ser Lys Gly Tyr Val Asn |     |     |     |     |
|    | 385   |     | 390 |     | 395 |
|    | Asn Val Glu Ala Thr Lys Glu Ala Ile Asp Asp Asp Gly Trp Leu His |     |     |     |     |
|    |   | 405 |     | 410 | 415 |
| 10 | Ser Gly Asp Phe Gly Tyr Tyr Asp Glu Asp Glu His Phe Tyr Val Val |     |     |     |     |
|    | 420   |     | 425 |     | 430 |
|    | Asp Arg Tyr Lys Glu Leu Ile Lys Tyr Lys Gly Ser Gln Val Ala Pro |     |     |     |     |
|    | 435   |     | 440 |     | 445 |
|    | Ala Glu Leu Glu Glu Ile Leu Leu Lys Asn Pro Cys Ile Arg Asp Val |     |     |     |     |
| 15 | 450   |     | 455 |     | 460 |
|    | Ala Val Val Gly Ile Pro Asp Leu Glu Ala Gly Glu Leu Pro Ser Ala |     |     |     |     |
|    | 465   |     | 470 |     | 475 |
|    | Phe Val Val Lys Gln Pro Gly Lys Glu Ile Thr Ala Lys Glu Val Tyr |     |     |     |     |
|    |   | 485 |     | 490 | 495 |
| 20 | Asp Tyr Leu Ala Glu Arg Val Ser His Thr Lys Tyr Leu Arg Gly Gly |     |     |     |     |
|    | 500   |     | 505 |     | 510 |
|    | Val Arg Phe Val Asp Ser Ile Pro Arg Asn Val Thr Gly Lys Ile Thr |     |     |     |     |
|    | 515   |     | 520 |     | 525 |
|    | Arg Lys Glu Leu Leu Lys Gln Leu Leu Glu Lys Ala Gly Gly         |     |     |     |     |
| 25 | 530   |     | 535 |     | 540 |

&lt;210&gt; 27

&lt;211&gt; 542

&lt;212&gt; PRT

30&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Sequence of a synthetic luciferase

35&lt;400&gt; 27

Met Met Lys Arg Glu Lys Asn Val Ile Tyr Gly Pro Glu Pro Leu His

1

5

10

15

Pro Leu Glu Asp Leu Thr Ala Gly Glu Met Leu Phe Arg Ala Leu Arg

20

25

30

40Lys His Ser His Leu Pro Gln Ala Leu Val Asp Val Val Gly Asp Glu

|       |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|       | 35  |     | 40  |     | 45  |     |     |     |     |     |     |     |     |     |     |     |
|       | Ser | Leu | Ser | Tyr | Lys | Glu | Phe | Phe | Glu | Ala | Thr | Val | Leu | Leu | Ala | Gln |
|       | 50  |     |     |     | 55  |     |     |     |     |     |     | 60  |     |     |     |     |
|       | Ser | Leu | His | Asn | Cys | Gly | Tyr | Lys | Met | Asn | Asp | Val | Val | Ser | Ile | Cys |
| 565   |     |     |     |     | 70  |     |     |     |     | 75  |     |     |     |     | 80  |     |
|       | Ala | Glu | Asn | Asn | Thr | Arg | Phe | Phe | Ile | Pro | Val | Ile | Ala | Ala | Trp | Tyr |
|       |     |     |     | 85  |     |     |     |     |     | 90  |     |     |     |     | 95  |     |
|       | Ile | Gly | Met | Ile | Val | Ala | Pro | Val | Asn | Glu | Ser | Tyr | Ile | Pro | Asp | Glu |
|       |     |     |     | 100 |     |     |     |     | 105 |     |     |     |     | 110 |     |     |
| 10    | Leu | Cys | Lys | Val | Met | Gly | Ile | Ser | Lys | Pro | Gln | Ile | Val | Phe | Thr | Thr |
|       |     |     |     | 115 |     |     |     |     | 120 |     |     |     |     | 125 |     |     |
|       | Lys | Asn | Ile | Leu | Asn | Lys | Val | Leu | Glu | Val | Gln | Ser | Arg | Thr | Asn | Phe |
|       |     |     |     | 130 |     |     |     |     | 135 |     |     |     |     | 140 |     |     |
|       | Ile | Lys | Arg | Ile | Ile | Ile | Leu | Asp | Thr | Val | Glu | Asn | Ile | His | Gly | Cys |
| 15145 |     |     |     |     | 150 |     |     |     |     | 155 |     |     |     |     | 160 |     |
|       | Glu | Ser | Leu | Pro | Asn | Phe | Ile | Ser | Arg | Tyr | Ser | Asp | Gly | Asn | Ile | Ala |
|       |     |     |     |     | 165 |     |     |     |     | 170 |     |     |     |     | 175 |     |
|       | Asn | Phe | Lys | Pro | Leu | His | Phe | Asp | Pro | Val | Glu | Gln | Val | Ala | Ala | Ile |
|       |     |     |     | 180 |     |     |     |     | 185 |     |     |     |     |     | 190 |     |
| 20    | Leu | Cys | Ser | Ser | Gly | Thr | Thr | Gly | Leu | Pro | Lys | Gly | Val | Met | Gln | Thr |
|       |     |     |     | 195 |     |     |     |     | 200 |     |     |     |     | 205 |     |     |
|       | His | Gln | Asn | Ile | Cys | Val | Arg | Leu | Ile | His | Ala | Leu | Asp | Pro | Arg | Val |
|       |     |     |     | 210 |     |     |     |     | 215 |     |     |     |     | 220 |     |     |
|       | Gly | Thr | Gln | Leu | Ile | Pro | Gly | Val | Thr | Val | Leu | Val | Tyr | Leu | Pro | Phe |
| 25225 |     |     |     |     | 230 |     |     |     |     | 235 |     |     |     |     | 240 |     |
|       | Phe | His | Ala | Phe | Gly | Phe | Ser | Ile | Thr | Leu | Gly | Tyr | Phe | Met | Val | Gly |
|       |     |     |     | 245 |     |     |     |     |     | 250 |     |     |     |     | 255 |     |
|       | Leu | Arg | Val | Ile | Met | Phe | Arg | Arg | Phe | Asp | Gln | Glu | Ala | Phe | Leu | Lys |
|       |     |     |     | 260 |     |     |     |     | 265 |     |     |     |     |     | 270 |     |
| 30    | Ala | Ile | Gln | Asp | Tyr | Glu | Val | Arg | Ser | Val | Ile | Asn | Val | Pro | Ser | Val |
|       |     |     |     | 275 |     |     |     |     | 280 |     |     |     |     | 285 |     |     |
|       | Ile | Leu | Phe | Leu | Ser | Lys | Ser | Pro | Leu | Val | Asp | Lys | Tyr | Asp | Leu | Ser |
|       |     |     |     | 290 |     |     |     |     | 295 |     |     |     |     | 300 |     |     |
|       | Ser | Leu | Arg | Glu | Leu | Cys | Cys | Gly | Ala | Ala | Pro | Leu | Ala | Lys | Glu | Val |
| 35305 |     |     |     |     | 310 |     |     |     |     |     | 315 |     |     |     | 320 |     |
|       | Ala | Glu | Val | Ala | Ala | Lys | Arg | Leu | Asn | Leu | Pro | Gly | Ile | Arg | Cys | Gly |
|       |     |     |     | 325 |     |     |     |     |     | 330 |     |     |     |     | 335 |     |
|       | Phe | Gly | Leu | Thr | Glu | Ser | Thr | Ser | Ala | Asn | Ile | His | Ser | Leu | Arg | Asp |
|       |     |     |     | 340 |     |     |     |     | 345 |     |     |     |     |     | 350 |     |
| 40    | Glu | Phe | Lys | Ser | Gly | Ser | Leu | Gly | Arg | Val | Thr | Pro | Leu | Met | Ala | Ala |

30

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          355              360              365
Lys Ile Ala Asp Arg Glu Thr Gly Lys Ala Leu Gly Pro Asn Gln Val
          370              375              380
----- Gly Glu Leu Cys Ile Lys Gly Pro Met Val Ser Lys Gly Tyr Val Asn -----
5385              390              395              400
Asn Val Glu Ala Thr Lys Glu Ala Ile Asp Asp Asp Gly Trp Leu His
          405              410              415
Ser Gly Asp Phe Gly Tyr Tyr Asp Glu Asp Glu His Phe Tyr Val Val
          420              425              430
10Asp Arg Tyr Lys Glu Leu Ile Lys Tyr Lys Gly Ser Gln Val Ala Pro
          435              440              445
Ala Glu Leu Glu Glu Ile Leu Leu Lys Asn Pro Cys Ile Arg Asp Val
          450              455              460
Ala Val Val Gly Ile Pro Asp Leu Glu Ala Gly Glu Leu Pro Ser Ala
15465              470              475              480
Phe Val Val Lys Gln Pro Gly Lys Glu Ile Thr Ala Lys Glu Val Tyr
          485              490              495
Asp Tyr Leu Ala Glu Arg Val Ser His Thr Lys Tyr Leu Arg Gly Gly
          500              505              510
20Val Arg Phe Val Asp Ser Ile Pro Arg Asn Val Thr Gly Lys Ile Thr
          515              520              525
Arg Lys Glu Leu Leu Lys Gln Leu Leu Glu Lys Ala Gly Gly
          530              535              540

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25&lt;210&gt; 28

&lt;211&gt; 542

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

30&lt;220&gt;

&lt;223&gt; Sequence of a synthetic luciferase

&lt;400&gt; 28

```

Met Met Lys Arg Glu Lys Asn Val Ile Tyr Gly Pro Glu Pro Leu His
35 1              5              10              15
Pro Leu Glu Asp Leu Thr Ala Gly Glu Met Leu Phe Arg Ala Leu Arg
          20              25              30
Lys His Ser His Leu Pro Gln Ala Leu Val Asp Val Val Gly Asp Glu
          35              40              45
40Ser Leu Ser Tyr Lys Glu Phe Phe Glu Ala Thr Val Leu Leu Ala Gln

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31

|   |     |     |
|---|-----|-----|
| 50  | 55  | 60  |
| Ser Leu His Asn Cys Gly Tyr Lys Met Asn Asp Val Val Ser Ile Cys   |     |     |
| 65  | 70  | 75  |
| 80  |     |     |
| Ala Glu Asn Asn Thr Arg Phe Phe Ile Pro Val Ile Ala Ala Trp Tyr   |     |     |
| 5   | 85  | 90  |
| 95  |     |     |
| Ile Gly Met Ile Val Ala Pro Val Asn Glu Ser Tyr Ile Pro Asp Glu   |     |     |
| 100   | 105 | 110 |
| Leu Cys Lys Val Met Gly Ile Ser Lys Pro Gln Ile Val Phe Thr Thr   |     |     |
| 115   | 120 | 125 |
| 10Lys Asn Ile Leu Asn Lys Val Leu Glu Val Gln Ser Arg Thr Asn Phe |     |     |
| 130   | 135 | 140 |
| Ile Lys Arg Ile Ile Ile Leu Asp Thr Val Glu Asn Ile His Gly Cys   |     |     |
| 145   | 150 | 155 |
| 160   |     |     |
| Glu Ser Leu Pro Asn Phe Ile Ser Arg Tyr Ser Asp Gly Asn Ile Ala   |     |     |
| 15  | 165 | 170 |
| 175   |     |     |
| Asn Phe Lys Pro Leu His Phe Asp Pro Val Glu Gln Val Ala Ala Ile   |     |     |
| 180   | 185 | 190 |
| Leu Cys Ser Ser Gly Thr Thr Gly Leu Pro Lys Gly Val Met Gln Thr   |     |     |
| 195   | 200 | 205 |
| 20His Gln Asn Ile Cys Val Arg Leu Ile His Ala Leu Asp Pro Arg Val |     |     |
| 210   | 215 | 220 |
| Gly Thr Gln Leu Ile Pro Gly Val Thr Val Leu Val Tyr Leu Pro Phe   |     |     |
| 225   | 230 | 235 |
| 240   |     |     |
| Phe His Ala Phe Gly Phe Ser Ile Thr Leu Gly Tyr Phe Met Val Gly   |     |     |
| 25  | 245 | 250 |
| 255   |     |     |
| Leu Arg Val Ile Met Phe Arg Arg Phe Asp Gln Glu Ala Phe Leu Lys   |     |     |
| 260   | 265 | 270 |
| Ala Ile Gln Asp Tyr Glu Val Arg Ser Val Ile Asn Val Pro Ser Val   |     |     |
| 275   | 280 | 285 |
| 30Ile Leu Phe Leu Ser Lys Ser Pro Leu Val Asp Lys Tyr Asp Leu Ser |     |     |
| 290   | 295 | 300 |
| Ser Leu Arg Glu Leu Cys Cys Gly Ala Ala Pro Leu Ala Lys Glu Val   |     |     |
| 305   | 310 | 315 |
| 320   |     |     |
| Ala Glu Val Ala Ala Lys Arg Leu Asn Leu Pro Gly Ile Arg Cys Gly   |     |     |
| 35  | 325 | 330 |
| 335   |     |     |
| Phe Gly Leu Thr Glu Ser Thr Ser Ala Asn Ile His Ser Leu Arg Asp   |     |     |
| 340   | 345 | 350 |
| Glu Phe Lys Ser Gly Ser Leu Gly Arg Val Thr Pro Leu Met Ala Ala   |     |     |
| 355   | 360 | 365 |
| 40Lys Ile Ala Asp Arg Glu Thr Gly Lys Ala Leu Gly Pro Asn Gln Val |     |     |

32

370                                      375                                      380  
 Gly Glu Leu Cys Ile Lys Gly Pro Met Val Ser Lys Gly Tyr Val Asn  
 385                                      390                                      395                                      400  
 Asn Val Glu Ala Thr Lys Glu Ala Ile Asp Asp Asp Gly Trp Leu His  
 5                                      405                                      410                                      415  
 Ser Gly Asp Phe Gly Tyr Tyr Asp Glu Asp Glu His Phe Tyr Val Val  
                                     420                                      425                                      430  
 Asp Arg Tyr Lys Glu Leu Ile Lys Tyr Lys Gly Ser Gln Val Ala Pro  
                                     435                                      440                                      445  
 10Ala Glu Leu Glu Glu Ile Leu Leu Lys Asn Pro Cys Ile Arg Asp Val  
                                     450                                      455                                      460  
 Ala Val Val Gly Ile Pro Asp Leu Glu Ala Gly Glu Leu Pro Ser Ala  
 465                                      470                                      475                                      480  
 Phe Val Val Lys Gln Pro Gly Lys Glu Ile Thr Ala Lys Glu Val Tyr  
 15                                      485                                      490                                      495  
 Asp Tyr Leu Ala Glu Arg Val Ser His Thr Lys Tyr Leu Arg Gly Gly  
                                     500                                      505                                      510  
 Val Arg Phe Val Asp Ser Ile Pro Arg Asn Val Thr Gly Lys Ile Thr  
                                     515                                      520                                      525  
 20Arg Lys Glu Leu Leu Lys Gln Leu Leu Glu Lys Ala Gly Gly  
                                     530                                      535                                      540

&lt;210&gt; 29

&lt;211&gt; 542

25&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Sequence of a synthetic luciferase

30

&lt;400&gt; 29

Met Met Lys Arg Glu Lys Asn Val Ile Tyr Gly Pro Glu Pro Leu His  
 1                                      5                                      10                                      15  
 Pro Leu Glu Asp Leu Thr Ala Gly Glu Met Leu Phe Arg Ala Leu Arg  
 35                                      20                                      25                                      30  
 Lys His Ser His Leu Pro Gln Ala Leu Val Asp Val Val Gly Asp Glu  
                                     35                                      40                                      45  
 Ser Leu Ser Tyr Lys Glu Phe Phe Glu Ala Thr Val Leu Leu Ala Gln  
                                     50                                      55                                      60  
 40Ser Leu His Asn Cys Gly Tyr Lys Met Asn Asp Val Val Ser Ile Cys

33

|  |     |     |     |
|--|-----|-----|-----|
| 65   | 70  | 75  | 80  |
| Ala Glu Asn Asn Thr Arg Phe Phe Ile Pro Val Ile Ala Ala Trp Tyr    |     |     |     |
|  | 85  | 90  | 95  |
| Ile Gly Met Ile Val Ala Pro Val Asn Glu Ser Tyr Ile Pro Asp Glu    |     |     |     |
| 5  | 100 | 105 | 110 |
| Leu Cys Lys Val Met Gly Ile Ser Lys Pro Gln Ile Val Phe Thr Thr    |     |     |     |
|  | 115 | 120 | 125 |
| Lys Asn Ile Leu Asn Lys Val Leu Glu Val Gln Ser Arg Thr Asn Phe    |     |     |     |
|  | 130 | 135 | 140 |
| 10 Ile Lys Arg Ile Ile Ile Leu Asp Thr Val Glu Asn Ile His Gly Cys |     |     |     |
|  | 145 | 150 | 155 |
| Glu Ser Leu Pro Asn Phe Ile Ser Arg Tyr Ser Asp Gly Asn Ile Ala    |     |     |     |
|  | 165 | 170 | 175 |
| Asn Phe Lys Pro Leu His Phe Asp Pro Val Glu Gln Val Ala Ala Ile    |     |     |     |
| 15   | 180 | 185 | 190 |
| Leu Cys Ser Ser Gly Thr Thr Gly Leu Pro Lys Gly Val Met Gln Thr    |     |     |     |
|  | 195 | 200 | 205 |
| His Gln Asn Ile Cys Val Arg Leu Ile His Ala Leu Asp Pro Arg Val    |     |     |     |
|  | 210 | 215 | 220 |
| 20 Gly Thr Gln Leu Ile Pro Gly Val Thr Val Leu Val Tyr Leu Pro Phe |     |     |     |
|  | 225 | 230 | 235 |
| Phe His Ala Phe Gly Phe Ser Ile Thr Leu Gly Tyr Phe Met Val Gly    |     |     |     |
|  | 245 | 250 | 255 |
| Leu Arg Val Ile Met Phe Arg Arg Phe Asp Gln Glu Ala Phe Leu Lys    |     |     |     |
| 25   | 260 | 265 | 270 |
| Ala Ile Gln Asp Tyr Glu Val Arg Ser Val Ile Asn Val Pro Ser Val    |     |     |     |
|  | 275 | 280 | 285 |
| Ile Leu Phe Leu Ser Lys Ser Pro Leu Val Asp Lys Tyr Asp Leu Ser    |     |     |     |
|  | 290 | 295 | 300 |
| 30 Ser Leu Arg Glu Leu Cys Cys Gly Ala Ala Pro Leu Ala Lys Glu Val |     |     |     |
|  | 305 | 310 | 315 |
| Ala Glu Val Ala Ala Lys Arg Leu Asn Leu Pro Gly Ile Arg Cys Gly    |     |     |     |
|  | 325 | 330 | 335 |
| Phe Gly Leu Thr Glu Ser Thr Ser Ala Asn Ile His Ser Leu Arg Asp    |     |     |     |
| 35   | 340 | 345 | 350 |
| Glu Phe Lys Ser Gly Ser Leu Gly Arg Val Thr Pro Leu Met Ala Ala    |     |     |     |
|  | 355 | 360 | 365 |
| Lys Ile Ala Asp Arg Glu Thr Gly Lys Ala Leu Gly Pro Asn Gln Val    |     |     |     |
|  | 370 | 375 | 380 |
| 40 Gly Glu Leu Cys Ile Lys Gly Pro Met Val Ser Lys Gly Tyr Val Asn |     |     |     |

34

385                      390                      395                      400  
 Asn Val Glu Ala Thr Lys Glu Ala Ile Asp Asp Asp Gly Trp Leu His  
                          405                      410                      415  
 Ser Gly Asp Phe Gly Tyr Tyr Asp Glu Asp Glu His Phe Tyr Val Val  
 5                      420                      425                      430  
 Asp Arg Tyr Lys Glu Leu Ile Lys Tyr Lys Gly Ser Gln Val Ala Pro  
                          435                      440                      445  
 Ala Glu Leu Glu Glu Ile Leu Leu Lys Asn Pro Cys Ile Arg Asp Val  
                          450                      455                      460  
 10Ala Val Val Gly Ile Pro Asp Leu Glu Ala Gly Glu Leu Pro Ser Ala  
                          465                      470                      475                      480  
 Phe Val Val Lys Gln Pro Gly Lys Glu Ile Thr Ala Lys Glu Val Tyr  
                          485                      490                      495  
 Asp Tyr Leu Ala Glu Arg Val Ser His Thr Lys Tyr Leu Arg Gly Gly  
 15                      500                      505                      510  
 Val Arg Phe Val Asp Ser Ile Pro Arg Asn Val Thr Gly Lys Ile Thr  
                          515                      520                      525  
 Arg Lys Glu Leu Leu Lys Gln Leu Leu Glu Lys Ala Gly Gly  
                          530                      535                      540

20

&lt;210&gt; 30

&lt;211&gt; 542

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

25

&lt;220&gt;

&lt;223&gt; Sequence of a synthetic luciferase

&lt;400&gt; 30

30Met Met Lys Arg Glu Lys Asn Val Ile Tyr Gly Pro Glu Pro Leu His  
     1                      5                      10                      15  
 Pro Leu Glu Asp Leu Thr Ala Gly Glu Met Leu Phe Arg Ala Leu Arg  
                          20                      25                      30  
 Lys His Ser His Leu Pro Gln Ala Leu Val Asp Val Val Gly Asp Glu  
 35                      35                      40                      45  
 Asn Leu Ser Tyr Lys Glu Phe Phe Glu Ala Thr Val Leu Leu Ala Gln  
                          50                      55                      60  
 Ser Leu His Asn Cys Gly Tyr Lys Met Asn Asp Val Val Ser Ile Cys  
                          65                      70                      75                      80  
 40Ala Glu Asn Asn Thr Arg Phe Phe Ile Pro Val Ile Ala Ala Trp Tyr



35

|    | 85  | 90  | 95  |
|----|---|-----|-----|
|    | Ile Gly Met Ile Val Ala Pro Val Asn Glu Ser Tyr Ile Pro Asp Glu |     |     |
|    | 100   | 105 | 110 |
| 1  | Leu Cys Lys Val Met Gly Ile Ser Lys Pro Gln Ile Val Phe Thr Thr |     |     |
| 5  | 115   | 120 | 125 |
|    | Lys Asn Ile Leu Asn Lys Val Leu Glu Val Gln Ser Arg Thr Asn Phe |     |     |
|    | 130   | 135 | 140 |
|    | Ile Lys Arg Ile Ile Ile Leu Asp Thr Val Glu Asn Ile His Gly Cys |     |     |
|    | 145   | 150 | 155 |
| 10 | Glu Ser Leu Pro Asn Phe Ile Ser Arg Tyr Ser Asp Gly Asn Ile Ala |     |     |
|    | 165   | 170 | 175 |
|    | Asn Phe Lys Pro Leu His Phe Asp Pro Val Glu Gln Val Ala Ala Ile |     |     |
|    | 180   | 185 | 190 |
|    | Leu Cys Ser Ser Gly Thr Thr Gly Leu Pro Lys Gly Val Met Gln Thr |     |     |
| 15 | 195   | 200 | 205 |
|    | His Gln Asn Ile Cys Val Arg Leu Ile His Ala Leu Asp Pro Arg Val |     |     |
|    | 210   | 215 | 220 |
|    | Gly Thr Gln Leu Ile Ser Gly Val Thr Val Leu Val Tyr Leu Pro Phe |     |     |
|    | 225   | 230 | 235 |
| 20 | Phe His Ala Phe Gly Phe Ser Ile Thr Leu Gly Tyr Phe Met Val Gly |     |     |
|    | 245   | 250 | 255 |
|    | Leu Arg Val Ile Met Phe Arg Arg Phe Asp Gln Glu Ala Phe Leu Lys |     |     |
|    | 260   | 265 | 270 |
|    | Ala Ile Gln Asp Tyr Glu Val Arg Ser Val Ile Asn Val Pro Ser Val |     |     |
| 25 | 275   | 280 | 285 |
|    | Ile Leu Phe Leu Ser Lys Ser Pro Leu Val Asp Lys Tyr Asp Leu Ser |     |     |
|    | 290   | 295 | 300 |
|    | Ser Leu Arg Glu Leu Cys Cys Gly Ala Ala Pro Leu Ala Lys Glu Val |     |     |
|    | 305   | 310 | 315 |
| 30 | Ala Glu Val Ala Ala Lys Arg Leu Asn Leu Pro Gly Ile Arg Cys Gly |     |     |
|    | 325   | 330 | 335 |
|    | Phe Gly Leu Thr Glu Ser Thr Ser Ala Asn Ile His Ser Leu Arg Asp |     |     |
|    | 340   | 345 | 350 |
|    | Glu Phe Lys Ser Gly Ser Leu Gly Arg Val Thr Pro Leu Met Ala Ala |     |     |
| 35 | 355   | 360 | 365 |
|    | Lys Ile Ala Asp Arg Glu Thr Gly Lys Ala Leu Gly Pro Asn Gln Val |     |     |
|    | 370   | 375 | 380 |
|    | Gly Glu Leu Cys Ile Lys Gly Pro Met Val Ser Lys Gly Tyr Val Asn |     |     |
|    | 385   | 390 | 395 |
| 40 | Asn Val Glu Ala Thr Lys Glu Ala Ile Asp Asp Asp Gly Trp Leu His |     |     |

36

405                      410                      415

Ser Gly Asp Phe Gly Tyr Tyr Asp Glu Asp Glu His Phe Tyr Val Val

420                      425                      430

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Asp Arg Tyr Lys Glu Leu Ile Lys Tyr Lys Gly Ser Gln Val Ala Pro

5                      435                      440                      445

Ala Glu Leu Glu Glu Ile Leu Leu Lys Asn Pro Cys Ile Arg Asp Val

450                      455                      460

Ala Val Val Gly Ile Pro Asp Leu Glu Ala Gly Glu Leu Pro Ser Ala

465                      470                      475                      480

10Phe Val Val Lys Gln Pro Gly Lys Glu Ile Thr Ala Lys Glu Val Tyr

485                      490                      495

Asp Tyr Leu Ala Glu Arg Val Ser His Thr Lys Tyr Leu Arg Gly Gly

500                      505                      510

Val Arg Phe Val Asp Ser Ile Pro Arg Asn Val Thr Gly Lys Ile Thr

15                      515                      520                      525

Arg Lys Glu Leu Leu Lys Gln Leu Leu Glu Lys Ala Gly Gly

530                      535                      540

&lt;210&gt; 31

20&lt;211&gt; 542

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

25&lt;223&gt; Sequence of a synthetic luciferase

&lt;400&gt; 31

Met Met Lys Arg Glu Lys Asn Val Ile Tyr Gly Pro Glu Pro Leu His

1                      5                      10                      15

30Pro Leu Glu Asp Leu Thr Ala Gly Glu Met Leu Phe Arg Ala Leu Arg

20                      25                      30

Lys His Ser His Leu Pro Gln Ala Leu Val Asp Val Val Gly Asp Glu

35                      40                      45

Ser Leu Ser Tyr Lys Glu Phe Phe Glu Ala Thr Val Leu Leu Ala Gln

35                      50                      55                      60

Ser Leu His Asn Cys Gly Tyr Lys Met Asn Asp Val Val Ser Ile Cys

65                      70                      75                      80

Ala Glu Asn Asn Thr Arg Phe Phe Ile Pro Val Ile Ala Ala Trp Tyr

85                      90                      95

40Ile Gly Met Ile Val Ala Pro Val Asn Glu Ser Tyr Ile Pro Asp Glu

100 105 110  
 Leu Cys Lys Val Met Gly Ile Ser Lys Pro Gln Ile Val Phe Thr Thr  
 115 120 125  
 Lys Asn Ile Leu Asn Lys Val Leu Glu Val Gln Ser Arg Thr Asn Phe  
 5 130 135 140  
 Ile Lys Arg Ile Ile Ile Leu Asp Thr Val Glu Asn Ile His Gly Cys  
 145 150 155 160  
 Glu Ser Leu Pro Asn Phe Ile Ser Arg Tyr Ser Asp Gly Asn Ile Ala  
 165 170 175  
 10 Asn Phe Lys Pro Leu His Phe Asp Pro Val Glu Gln Val Ala Ala Ile  
 180 185 190  
 Leu Cys Ser Ser Gly Thr Thr Gly Leu Pro Lys Gly Val Met Gln Thr  
 195 200 205  
 His Gln Asn Ile Cys Val Arg Leu Ile His Ala Leu Asp Pro Arg Val  
 15 210 215 220  
 Gly Thr Gln Leu Ile Pro Gly Val Thr Val Leu Val Tyr Leu Pro Phe  
 225 230 235 240  
 Phe His Ala Phe Gly Phe Ser Ile Thr Leu Gly Tyr Phe Met Val Gly  
 245 250 255  
 20 Leu Arg Val Ile Met Phe Arg Arg Phe Asp Gln Glu Ala Phe Leu Lys  
 260 265 270  
 Ala Ile Gln Asp Tyr Glu Val Arg Ser Val Ile Asn Val Pro Ser Val  
 275 280 285  
 Ile Leu Phe Leu Ser Lys Ser Pro Leu Val Asp Lys Tyr Asp Leu Ser  
 25 290 295 300  
 Ser Leu Arg Glu Leu Cys Cys Gly Ala Ala Pro Leu Ala Lys Glu Val  
 305 310 315 320  
 Ala Glu Val Ala Ala Lys Arg Leu Asn Leu Pro Gly Ile Arg Cys Gly  
 325 330 335  
 30 Phe Gly Leu Thr Glu Ser Thr Ser Ala Asn Ile His Ser Leu Arg Asp  
 340 345 350  
 Glu Phe Lys Ser Gly Ser Leu Gly Arg Val Thr Pro Leu Met Ala Ala  
 355 360 365  
 Lys Ile Ala Asp Arg Glu Thr Gly Lys Ala Leu Gly Pro Asn Gln Val  
 35 370 375 380  
 Gly Glu Leu Cys Ile Lys Gly Pro Met Val Ser Lys Gly Tyr Val Asn  
 385 390 395 400  
 Asn Val Glu Ala Thr Lys Glu Ala Ile Asp Asp Asp Gly Trp Leu His  
 405 410 415  
 40 Ser Gly Asp Phe Gly Tyr Tyr Asp Glu Asp Glu His Phe Tyr Val Val

38

420                                      425                                      430  
 Asp Arg Tyr Lys Glu Leu Ile Lys Tyr Lys Gly Ser Gln Val Ala Pro  
    435                                      440                                      445  
 Ala Glu Leu Glu Glu Ile Leu Leu Lys Asn Pro Cys Ile Arg Asp Val  
 5        450                                      455                                      460  
 Ala Val Val Gly Ile Pro Asp Leu Glu Ala Gly Glu Leu Pro Ser Ala  
 465                                      470                                      475                                      480  
 Phe Val Val Lys Gln Pro Gly Lys Glu Ile Thr Ala Lys Glu Val Tyr  
    485                                      490                                      495  
 10 Asp Tyr Leu Ala Glu Arg Val Ser His Thr Lys Tyr Leu Arg Gly Gly  
    500                                      505                                      510  
 Val Arg Phe Val Asp Ser Ile Pro Arg Asn Val Thr Gly Lys Ile Thr  
    515                                      520                                      525  
 Arg Lys Glu Leu Leu Lys Gln Leu Leu Glu Lys Ala Gly Gly  
 15        530                                      535                                      540

&lt;210&gt; 32

&lt;211&gt; 542

&lt;212&gt; PRT

20&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Sequence of a synthetic luciferase

25&lt;400&gt; 32

Met Met Lys Arg Glu Lys Asn Val Ile Tyr Gly Pro Glu Pro Leu His  
    1                                      5                                      10                                      15  
 Pro Leu Glu Asp Leu Thr Ala Gly Glu Met Leu Phe Arg Ala Leu Arg  
    20                                      25                                      30  
 30 Lys His Ser His Leu Pro Gln Ala Leu Val Asp Val Val Gly Asp Glu  
    35                                      40                                      45  
 Ser Leu Ser Tyr Lys Glu Phe Phe Glu Ala Thr Val Leu Leu Ala Gln  
    50                                      55                                      60  
 Ser Leu His Asn Cys Gly Tyr Lys Met Asn Asp Val Val Ser Ile Cys  
 35 65                                      70                                      75                                      80  
 Ala Glu Asn Asn Thr Arg Phe Phe Ile Pro Val Ile Ala Ala Trp Tyr  
    85                                      90                                      95  
 Ile Gly Met Ile Val Ala Pro Val Asn Glu Ser Tyr Ile Pro Asp Glu  
    100                                      105                                      110  
 40 Leu Cys Lys Val Met Gly Ile Ser Lys Pro Gln Ile Val Phe Thr Thr

|       | 115 |     |     |     |     | 120 |     |     |     |     | 125 |     |     |     |     |     |
|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|       | Lys | Asn | Ile | Leu | Asn | Lys | Val | Leu | Glu | Val | Gln | Ser | Arg | Thr | Asn | Phe |
|       | 130 |     |     |     |     | 135 |     |     |     |     | 140 |     |     |     |     |     |
| 1     | Ile | Lys | Arg | Ile | Ile | Ile | Leu | Asp | Thr | Val | Glu | Asn | Ile | His | Gly | Cys |
| 5145  | 150 |     |     |     |     | 155 |     |     |     |     | 160 |     |     |     |     |     |
|       | Glu | Ser | Leu | Pro | Asn | Phe | Ile | Ser | Arg | Tyr | Ser | Asp | Gly | Asn | Ile | Ala |
|       | 165 |     |     |     |     | 170 |     |     |     |     | 175 |     |     |     |     |     |
|       | Asn | Phe | Lys | Pro | Leu | His | Phe | Asp | Pro | Val | Glu | Gln | Val | Ala | Ala | Ile |
|       | 180 |     |     |     |     | 185 |     |     |     |     | 190 |     |     |     |     |     |
| 10    | Leu | Cys | Ser | Ser | Gly | Thr | Thr | Gly | Leu | Pro | Lys | Gly | Val | Met | Gln | Thr |
|       | 195 |     |     |     |     | 200 |     |     |     |     | 205 |     |     |     |     |     |
|       | His | Gln | Asn | Ile | Cys | Val | Arg | Leu | Ile | His | Ala | Leu | Asp | Pro | Arg | Tyr |
|       | 210 |     |     |     |     | 215 |     |     |     |     | 220 |     |     |     |     |     |
|       | Gly | Thr | Gln | Leu | Ile | Pro | Gly | Val | Thr | Val | Leu | Val | Tyr | Leu | Pro | Phe |
| 15225 | 230 |     |     |     |     | 235 |     |     |     |     | 240 |     |     |     |     |     |
|       | Phe | His | Ala | Phe | Gly | Phe | His | Ile | Thr | Leu | Gly | Tyr | Phe | Met | Val | Gly |
|       | 245 |     |     |     |     | 250 |     |     |     |     | 255 |     |     |     |     |     |
|       | Leu | Arg | Val | Ile | Met | Phe | Arg | Arg | Phe | Asp | Gln | Glu | Ala | Phe | Leu | Lys |
|       | 260 |     |     |     |     | 265 |     |     |     |     | 270 |     |     |     |     |     |
| 20    | Ala | Ile | Gln | Asp | Tyr | Glu | Val | Arg | Ser | Val | Ile | Asn | Val | Pro | Ser | Val |
|       | 275 |     |     |     |     | 280 |     |     |     |     | 285 |     |     |     |     |     |
|       | Ile | Leu | Phe | Leu | Ser | Lys | Ser | Pro | Leu | Val | Asp | Lys | Tyr | Asp | Leu | Ser |
|       | 290 |     |     |     |     | 295 |     |     |     |     | 300 |     |     |     |     |     |
|       | Ser | Leu | Arg | Glu | Leu | Cys | Cys | Gly | Ala | Ala | Pro | Leu | Ala | Lys | Glu | Val |
| 25305 | 310 |     |     |     |     | 315 |     |     |     |     | 320 |     |     |     |     |     |
|       | Ala | Glu | Val | Ala | Ala | Lys | Arg | Leu | Asn | Leu | Pro | Gly | Ile | Arg | Cys | Gly |
|       | 325 |     |     |     |     | 330 |     |     |     |     | 335 |     |     |     |     |     |
|       | Phe | Gly | Leu | Thr | Glu | Ser | Thr | Ser | Ala | Ile | Ile | Gln | Ser | Leu | Arg | Asp |
|       | 340 |     |     |     |     | 345 |     |     |     |     | 350 |     |     |     |     |     |
| 30    | Glu | Phe | Lys | Ser | Gly | Ser | Leu | Gly | Arg | Val | Thr | Pro | Leu | Met | Ala | Ala |
|       | 355 |     |     |     |     | 360 |     |     |     |     | 365 |     |     |     |     |     |
|       | Lys | Ile | Ala | Asp | Arg | Glu | Thr | Gly | Lys | Ala | Leu | Gly | Pro | Asn | Gln | Val |
|       | 370 |     |     |     |     | 375 |     |     |     |     | 380 |     |     |     |     |     |
|       | Gly | Glu | Leu | Cys | Ile | Lys | Gly | Pro | Met | Val | Ser | Lys | Gly | Tyr | Val | Asn |
| 35385 | 390 |     |     |     |     | 395 |     |     |     |     | 400 |     |     |     |     |     |
|       | Asn | Val | Glu | Ala | Thr | Lys | Glu | Ala | Ile | Asp | Asp | Asp | Gly | Trp | Leu | His |
|       | 405 |     |     |     |     | 410 |     |     |     |     | 415 |     |     |     |     |     |
|       | Ser | Gly | Asp | Phe | Gly | Tyr | Tyr | Asp | Glu | Asp | Glu | His | Phe | Tyr | Val | Val |
|       | 420 |     |     |     |     | 425 |     |     |     |     | 430 |     |     |     |     |     |
| 40    | Asp | Arg | Tyr | Lys | Glu | Leu | Ile | Lys | Tyr | Lys | Gly | Ser | Gln | Val | Ala | Pro |

40

|   |     |     |
|---|-----|-----|
| 435   | 440 | 445 |
| Ala Glu Leu Glu Glu Ile Leu Leu Lys Asn Pro Cys Ile Arg Asp Val   |     |     |
| 450   | 455 | 460 |
| Ala Val Val Gly Ile Pro Asp Leu Glu Ala Gly Glu Leu Pro Ser Ala   |     |     |
| 5465  | 470 | 475 |
| Phe Val Val Lys Gln Pro Gly Lys Glu Ile Thr Ala Lys Glu Val Tyr   |     |     |
|   | 485 | 490 |
| Asp Tyr Leu Ala Glu Arg Val Ser His Thr Lys Tyr Leu Arg Gly Gly   |     |     |
|   | 500 | 505 |
| 10Val Arg Phe Val Asp Ser Ile Pro Arg Asn Val Thr Gly Lys Ile Thr |     |     |
|   | 515 | 520 |
| Arg Lys Glu Leu Leu Lys Gln Leu Leu Glu Lys Ala Gly Gly           |     |     |
|   | 530 | 535 |
|   |     | 540 |

15&lt;210&gt; 33

&lt;211&gt; 542

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

20&lt;220&gt;

&lt;223&gt; Sequence of a synthetic luciferase

&lt;400&gt; 33

|   |     |     |
|---|-----|-----|
| Met Met Lys Arg Glu Lys Asn Val Ile Tyr Gly Pro Glu Pro Leu His   |     |     |
| 25 1  | 5   | 10  |
| Pro Leu Glu Asp Leu Thr Ala Gly Glu Met Leu Phe Arg Ala Leu Arg   |     |     |
|   | 20  | 25  |
| Lys His Ser His Leu Pro Gln Ala Leu Val Asp Val Val Gly Asp Glu   |     |     |
|   | 35  | 40  |
| 30Ser Leu Ser Tyr Lys Glu Phe Phe Glu Ala Thr Val Leu Leu Ala Gln |     |     |
|   | 50  | 55  |
| Ser Leu His Asn Cys Gly Tyr Lys Met Asn Asp Val Val Ser Ile Cys   |     |     |
|   | 65  | 70  |
| Ala Glu Asn Asn Thr Arg Phe Phe Ile Pro Val Ile Ala Ala Trp Tyr   |     |     |
| 35  | 85  | 90  |
| Ile Gly Met Ile Val Ala Pro Val Asn Glu Ser Tyr Ile Pro Asp Glu   |     |     |
|   | 100 | 105 |
| Leu Cys Lys Val Met Gly Ile Ser Lys Pro Gln Ile Val Phe Thr Thr   |     |     |
|   | 115 | 120 |
| 40Lys Asn Ile Leu Asn Lys Val Leu Glu Val Gln Ser Arg Thr Asn Phe |     |     |
|   |     | 125 |

|    |   |   |     |     |     |     |
|----|---|---|-----|-----|-----|-----|
|    | 130   |   | 135 |     | 140 |     |
|    | Ile Lys Arg Ile Ile Ile   | Leu Asp Thr Val Glu Asn Ile His Gly Cys |     |     |     |     |
|    | 145   |   | 150 |     | 155 | 160 |
|    | Glu Ser Leu Pro Asn Phe   | Ile Ser Arg Tyr Ser Asp Gly Asn Ile Ala |     |     |     |     |
| 5  |   | 165                                     |     | 170 |     | 175 |
|    | Asn Phe Lys Pro Leu His Phe Asp Pro Val Glu Gln Val Ala Ala Ile |   |     |     |     |     |
|    |   | 180                                     |     | 185 |     | 190 |
|    | Leu Cys Ser Ser Gly Thr Thr Gly Leu Pro Lys Gly Val Met Gln Thr |   |     |     |     |     |
|    | 195   |   | 200 |     | 205 |     |
| 10 | His Gln Asn Ile Cys Val Arg Leu Ile His Ala Leu Asp Pro Arg Tyr |   |     |     |     |     |
|    | 210   |   | 215 |     | 220 |     |
|    | Gly Thr Gln Leu Ile Pro Gly Val Thr Val Leu Val Tyr Leu Pro Phe |   |     |     |     |     |
|    | 225   |   | 230 |     | 235 | 240 |
|    | Phe His Ala Phe Gly Phe His Ile Thr Leu Gly Tyr Phe Met Val Gly |   |     |     |     |     |
| 15 |   | 245                                     |     | 250 |     | 255 |
|    | Leu Arg Val Ile Met Phe Arg Arg Phe Asp Gln Glu Ala Phe Leu Lys |   |     |     |     |     |
|    |   | 260                                     |     | 265 |     | 270 |
|    | Ala Ile Gln Asp Tyr Glu Val Arg Ser Val Ile Asn Val Pro Ser Val |   |     |     |     |     |
|    | 275   |   | 280 |     | 285 |     |
| 20 | Ile Leu Phe Leu Ser Lys Ser Pro Leu Val Asp Lys Tyr Asp Leu Ser |   |     |     |     |     |
|    | 290   |   | 295 |     | 300 |     |
|    | Ser Leu Arg Glu Leu Cys Cys Gly Ala Ala Pro Leu Ala Lys Glu Val |   |     |     |     |     |
|    | 305   |   | 310 |     | 315 | 320 |
|    | Ala Glu Val Ala Ala Lys Arg Leu Asn Leu Pro Gly Ile Arg Cys Gly |   |     |     |     |     |
| 25 |   | 325                                     |     | 330 |     | 335 |
|    | Phe Gly Leu Thr Glu Ser Thr Ser Ala Ile Ile Gln Ser Leu Arg Asp |   |     |     |     |     |
|    |   | 340                                     |     | 345 |     | 350 |
|    | Glu Phe Lys Ser Gly Ser Leu Gly Arg Val Thr Pro Leu Met Ala Ala |   |     |     |     |     |
|    | 355   |   | 360 |     | 365 |     |
| 30 | Lys Ile Ala Asp Arg Glu Thr Gly Lys Ala Leu Gly Pro Asn Gln Val |   |     |     |     |     |
|    | 370   |   | 375 |     | 380 |     |
|    | Gly Glu Leu Cys Ile Lys Gly Pro Met Val Ser Lys Gly Tyr Val Asn |   |     |     |     |     |
|    | 385   |   | 390 |     | 395 | 400 |
|    | Asn Val Glu Ala Thr Lys Glu Ala Ile Asp Asp Asp Gly Trp Leu His |   |     |     |     |     |
| 35 |   | 405                                     |     | 410 |     | 415 |
|    | Ser Gly Asp Phe Gly Tyr Tyr Asp Glu Asp Glu His Phe Tyr Val Val |   |     |     |     |     |
|    |   | 420                                     |     | 425 |     | 430 |
|    | Asp Arg Tyr Lys Glu Leu Ile Lys Tyr Lys Gly Ser Gln Val Ala Pro |   |     |     |     |     |
|    | 435   |   | 440 |     | 445 |     |
| 40 | Ala Glu Leu Glu Glu Ile Leu Leu Lys Asn Pro Cys Ile Arg Asp Val |   |     |     |     |     |

42

```

      450              455              460
Ala Val Val Gly Ile Pro Asp Leu Glu Ala Gly Glu Leu Pro Ser Ala
465              470              475              480
Phe Val Val Lys Gln Pro Gly Lys Glu Ile Thr Ala Lys Glu Val Tyr
5              485              490              495
Asp Tyr Leu Ala Glu Arg Val Ser His Thr Lys Tyr Leu Arg Gly Gly
              500              505              510
Val Arg Phe Val Asp Ser Ile Pro Arg Asn Val Thr Gly Lys Ile Thr
              515              520              525
10Arg Lys Glu Leu Leu Lys Gln Leu Leu Glu Lys Ala Gly Gly
              530              535              540

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&lt;210&gt; 34

&lt;211&gt; 542

15&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Sequence of a synthetic luciferase

20

&lt;400&gt; 34

```

Met Met Lys Arg Glu Lys Asn Val Ile Tyr Gly Pro Glu Pro Leu His
1              5              10              15
Pro Leu Glu Asp Leu Thr Ala Gly Glu Met Leu Phe Arg Ala Leu Arg
25              20              25              30
Lys His Ser His Leu Pro Gln Ala Leu Val Asp Val Val Gly Asp Glu
              35              40              45
Ser Leu Ser Tyr Lys Glu Phe Phe Glu Ala Thr Val Leu Leu Ala Gln
              50              55              60
30Ser Leu His Asn Cys Gly Tyr Lys Met Asn Asp Val Val Ser Ile Cys
65              70              75              80
Ala Glu Asn Asn Thr Arg Phe Phe Ile Pro Val Ile Ala Ala Trp Tyr
              85              90              95
Ile Gly Met Ile Val Ala Pro Val Asn Glu Ser Tyr Ile Pro Asp Glu
35              100              105              110
Leu Cys Lys Val Met Gly Ile Ser Lys Pro Gln Ile Val Phe Thr Thr
              115              120              125
Lys Asn Ile Leu Asn Lys Val Leu Glu Val Gln Ser Arg Thr Asn Phe
              130              135              140
40Ile Lys Arg Ile Ile Ile Leu Asp Thr Val Glu Asn Ile His Gly Cys

```



43

145                      150                      155                      160  
 Glu Ser Leu Pro Asn Phe Ile Ser Arg Tyr Ser Asp Gly Asn Ile Ala  
                          165                      170                      175  
 Asn Phe Lys Pro Leu His Phe Asp Pro Val Glu Gln Val Ala Ala Ile  
 5                      180                      185                      190  
 Leu Cys Ser Ser Gly Thr Thr Gly Leu Pro Lys Gly Val Met Gln Thr  
                          195                      200                      205  
 His Gln Asn Ile Cys Val Arg Leu Ile His Ala Leu Asp Pro Arg Tyr  
                          210                      215                      220  
 10Gly Thr Gln Leu Ile Pro Gly Val Thr Val Leu Val Tyr Leu Pro Phe  
                          225                      230                      235                      240  
 Phe His Ala Phe Gly Phe His Ile Thr Leu Gly Tyr Phe Met Val Gly  
                          245                      250                      255  
 Leu Arg Val Ile Met Phe Arg Arg Phe Asp Gln Glu Ala Phe Leu Lys  
 15                      260                      265                      270  
 Ala Ile Gln Asp Tyr Glu Val Arg Ser Val Ile Asn Val Pro Ser Val  
                          275                      280                      285  
 Ile Leu Phe Leu Ser Lys Ser Pro Leu Val Asp Lys Tyr Asp Leu Ser  
                          290                      295                      300  
 20Ser Leu Arg Glu Leu Cys Cys Gly Ala Ala Pro Leu Ala Lys Glu Val  
                          305                      310                      315                      320  
 Ala Glu Val Ala Ala Lys Arg Leu Asn Leu Pro Gly Ile Arg Cys Gly  
                          325                      330                      335  
 Phe Gly Leu Thr Glu Ser Thr Ser Ala Ile Ile Gln Ser Leu Arg Asp  
 25                      340                      345                      350  
 Glu Phe Lys Ser Gly Ser Leu Gly Arg Val Thr Pro Leu Met Ala Ala  
                          355                      360                      365  
 Lys Ile Ala Asp Arg Glu Thr Gly Lys Ala Leu Gly Pro Asn Gln Val  
                          370                      375                      380  
 30Gly Glu Leu Cys Ile Lys Gly Pro Met Val Ser Lys Gly Tyr Val Asn  
                          385                      390                      395                      400  
 Asn Val Glu Ala Thr Lys Glu Ala Ile Asp Asp Asp Gly Trp Leu His  
                          405                      410                      415  
 Ser Gly Asp Phe Gly Tyr Tyr Asp Glu Asp Glu His Phe Tyr Val Val  
 35                      420                      425                      430  
 Asp Arg Tyr Lys Glu Leu Ile Lys Tyr Lys Gly Ser Gln Val Ala Pro  
                          435                      440                      445  
 Ala Glu Leu Glu Glu Ile Leu Leu Lys Asn Pro Cys Ile Arg Asp Val  
                          450                      455                      460  
 40Ala Val Val Gly Ile Pro Asp Leu Glu Ala Gly Glu Leu Pro Ser Ala

44

465                      470                      475                      480  
 Phe Val Val Lys Gln Pro Gly Lys Glu Ile Thr Ala Lys Glu Val Tyr  
                          485                      490                      495  
 Asp Tyr Leu Ala Glu Arg Val Ser His Thr Lys Tyr Leu Arg Gly Gly  
 5                      500                      505                      510  
 Val Arg Phe Val Asp Ser Ile Pro Arg Asn Val Thr Gly Lys Ile Thr  
                          515                      520                      525  
 Arg Lys Glu Leu Leu Lys Gln Leu Leu Glu Lys Ala Gly Gly  
                          530                      535                      540

10

<210> 35  
 <211> 29  
 <212> DNA  
 <213> Artificial Sequence

15

<220>  
 <223> An oligonucleotide

&lt;400&gt; 35

20acgccagccc aagcttaggc ctgagtggc

29

&lt;210&gt; 36

&lt;211&gt; 44

&lt;212&gt; DNA

25&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; An oligonucleotide

30&lt;400&gt; 36

cttaattctc cccatcccc tgttgacaat taatcatcgg ctcg

44

&lt;210&gt; 37

&lt;211&gt; 40

35&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; An oligonucleotide

40

45

&lt;400&gt; 37

tataatgtga ggaattgcga gcggataaca atttcacaca

40

&lt;210&gt; 38

5&lt;211&gt; 40

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

10&lt;223&gt; An oligonucleotide

&lt;400&gt; 38

atgggatgtt acctagacca atatgaaata tttggtaa

40

15&lt;210&gt; 39

&lt;211&gt; 40

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

20&lt;220&gt;

&lt;223&gt; An oligonucleotide

&lt;400&gt; 39

aaatgcttaa tgaatttcaa aaaaaaaaaa aaaggaattc

40

25

&lt;210&gt; 40

&lt;211&gt; 40

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

30

&lt;220&gt;

&lt;223&gt; An oligonucleotide

&lt;400&gt; 40

35gatatcaagc ttatcgatac cgtcgacctc gaggattata

40

&lt;210&gt; 41

&lt;211&gt; 37

&lt;212&gt; DNA

40&lt;213&gt; Artificial Sequence

<220>

<223> An oligonucleotide

<400> 41

5tagaaaaagg cctcggcggc cgctagttca gtcagtt

37

<210> 42

<211> 17

<212> DNA

10<213> Artificial Sequence

<220>

<223> An oligonucleotide

15<400> 42

aactgactga actagcg

17

<210> 43

<211> 40

20<212> DNA

<213> Artificial Sequence

<220>

<223> An oligonucleotide

25

<400> 43

gccgccgagg cctttttcta tataatcctc gaggtcgacg

40

<210> 44

30<211> 40

<212> DNA

<213> Artificial Sequence

<220>

35<223> An oligonucleotide

<400> 44

gtatcgataa gcttgatata gaattccttt tttttttttt

40

40<210> 45

<211> 40

<212> DNA

<213> Artificial Sequence

5<220>

<223> An oligonucleotide

<400> 45

agcttgatat cgaattcctt tttttttttt tttgaaattc

40

10

<210> 46

<211> 40

<212> DNA

<213> Artificial Sequence

15

<220>

<223> An oligonucleotide

<400> 46

20ttgaaattca ttaagcattt atttaccaaa tatttcatat

40

<210> 47

<211> 40

<212> DNA

25<213> Artificial Sequence

<220>

<223> An oligonucleotide

30<400> 47

tggtctaggt aacatcccat cactagcttt tttttctata

40

<210> 48

<211> 40

35<212> DNA

<213> Artificial Sequence

<220>

<223> An oligonucleotide

40

48

&lt;400&gt; 48

tcgcaattcc tcacattata cgagccgatg attaatgtc

40

&lt;210&gt; 49

5&lt;211&gt; 53

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

10&lt;223&gt; An oligonucleotide

&lt;400&gt; 49

aacaggggga tggggagaat taaggccact caggcctaag cttgggctgg cgt

53

15&lt;210&gt; 50

&lt;211&gt; 40

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

20&lt;220&gt;

&lt;223&gt; An oligonucleotide

&lt;400&gt; 50

ggaaacagga tcccatgatg aaacgcgaaa agaacgtgat

40

25

&lt;210&gt; 51

&lt;211&gt; 40

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

30

&lt;220&gt;

&lt;223&gt; An oligonucleotide

&lt;400&gt; 51

35ctacggccca gaaccactgc atccactgga agacctcacc

40

&lt;210&gt; 52

&lt;211&gt; 40

&lt;212&gt; DNA

40&lt;213&gt; Artificial Sequence

<220>

<223> An oligonucleotide

<400> 52

5gctggtgaga tgctcttccg agcactgcgt aaacatagtc

40

<210> 53

<211> 40

<212> DNA

10<213> Artificial Sequence

<220>

<223> An oligonucleotide

15<400> 53

acctccctca agcactcgtg gacgtcgtgg gagacgagag

40

<210> 54

<211> 40

20<212> DNA

<213> Artificial Sequence

<220>

<223> An oligonucleotide

25

<400> 54

cctctcctac aaagaatttt tcgaagctac tgtgctggtg

40

<210> 55

30<211> 40

<212> DNA

<213> Artificial Sequence

<220>

35<223> An oligonucleotide

<400> 55

gcccaaagcc tccataattg tgggtacaaa atgaacgatg

40

40<210> 56

50

&lt;211&gt; 40

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

5&lt;220&gt;

&lt;223&gt; An oligonucleotide

&lt;400&gt; 56

tgggtgagcat ttgtgctgag aataacactc gcttctttat

40

10

&lt;210&gt; 57

&lt;211&gt; 40

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

15

&lt;220&gt;

&lt;223&gt; An oligonucleotide

&lt;400&gt; 57

20tcctgtaatc gctgcttggt acatcggcac gattgtcgcc

40

&lt;210&gt; 58

&lt;211&gt; 40

&lt;212&gt; DNA

25&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; An oligonucleotide

30&lt;400&gt; 58

cctgtgaatg aatcttacat ccagatgag ctgtgtaagg

40

&lt;210&gt; 59

&lt;211&gt; 40

35&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; An oligonucleotide

40



<400> 59

ttatgggtat tagcaaact caaatcgtct ttactacaa

40

<210> 60

5<211> 40

<212> DNA

<213> Artificial Sequence

<220>

10<223> An oligonucleotide

<400> 60

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15<210> 61

<211> 40

<212> DNA

<213> Artificial Sequence

20<220>

<223> An oligonucleotide

<400> 61

aacttcacatca aacgcacatcat tattctggat accgtcgaaa

40

25

<210> 62

<211> 40

<212> DNA

<213> Artificial Sequence

30

<220>

<223> An oligonucleotide

<400> 62

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40

<210> 63

<211> 40

<212> DNA

40<213> Artificial Sequence

<220>

<223> An oligonucleotide

<400> 63

5ttacagcgat ggtaatatcg ctaatttcaa gccottgcat

40

<210> 64

<211> 40

<212> DNA

10<213> Artificial Sequence

<220>

<223> An oligonucleotide

15<400> 64

tttgatccag tcgagcaagt ggccgctatt ttgtgctcct

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<210> 65

<211> 40

20<212> DNA

<213> Artificial Sequence

<220>

<223> An oligonucleotide

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<400> 65

ccggcaccac tggtttgcct aaaggtgtca tgcagactca

40

<210> 66

30<211> 40

<212> DNA

<213> Artificial Sequence

<220>

35<223> An oligonucleotide

<400> 66

ccagaatatc tgtgtgcggt tgatccacgc tctcgaccct

40

40<210> 67

<211> 40

<212> DNA

<213> Artificial Sequence

5<220>

<223> An oligonucleotide

<400> 67

cgtgtgggta ctcaattgat ccctggcgtg actgtgctgg

40

10

<210> 68

<211> 40

<212> DNA

<213> Artificial Sequence

15

<220>

<223> An oligonucleotide

<400> 68

20tgtatctgcc tttctttcac gcctttggtt tctctattac

40

<210> 69

<211> 40

<212> DNA

25<213> Artificial Sequence

<220>

<223> An oligonucleotide

30<400> 69

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<210> 70

<211> 40

35<212> DNA

<213> Artificial Sequence

<220>

<223> An oligonucleotide

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<400> 70

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<210> 71

5<211> 40

<212> DNA

<213> Artificial Sequence

<220>

10<223> An oligonucleotide

<400> 71

actacgaggt gcggtccgtg atcaacgtcc cttcagtcac

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15<210> 72

<211> 43

<212> DNA

<213> Artificial Sequence

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<223> An oligonucleotide

<400> 72

tttggttcctg agcaaattctc ctttggttga caagtatgat ctg

43

25

<210> 73

<211> 37

<212> DNA

<213> Artificial Sequence

30

<220>

<223> An oligonucleotide

<400> 73

35agcagcttgc gtgagctgtg ctgtggcgct gctcctt

37

<210> 74

<211> 40

<212> DNA

40<213> Artificial Sequence

<220>

<223> An oligonucleotide

<400> 74

5tggccaaaga agtggccgag gtcgctgcta agcgtctgaa

40

<210> 75

<211> 40

<212> DNA

10<213> Artificial Sequence

<220>

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10&lt;223&gt; An oligonucleotide

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<220>

35<223> An oligonucleotide

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<211> 40

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<213> Artificial Sequence

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<400> 177

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<210> 178

<211> 40

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<220>

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<400> 178

20gttacgaggg atggagtcaa caaaacgcac gccgccacgc

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<210> 179

<211> 40

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25<213> Artificial Sequence

<220>

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aagtacttag tatgggtcac acgttcagcc aggtaatcat

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<210> 180

<211> 40

35<212> DNA

<213> Artificial Sequence

<220>

<223> An oligonucleotide

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&lt;210&gt; 181

5&lt;211&gt; 40

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

10&lt;223&gt; An oligonucleotide

&lt;400&gt; 181

aacgaaagca gaaggcagtt cgccggcctc cagatcagga

40

15&lt;210&gt; 182

&lt;211&gt; 40

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

20&lt;220&gt;

&lt;223&gt; An oligonucleotide

&lt;400&gt; 182

atgccgacca cagcgacatc gcgaatgcat ggatttttca

40

25

&lt;210&gt; 183

&lt;211&gt; 40

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

30

&lt;220&gt;

&lt;223&gt; An oligonucleotide

&lt;400&gt; 183

35acagaatctc ctccaactca gctggagcaa cctggctacc

40

&lt;210&gt; 184

&lt;211&gt; 40

&lt;212&gt; DNA

40&lt;213&gt; Artificial Sequence

<220>

<223> An oligonucleotide

<400> 184

5cttggtatttg atcagctcct tgtaacgatc cacgacgtaa

40

<210> 185

<211> 40

<212> DNA

10<213> Artificial Sequence

<220>

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15<400> 185

aaatggtcat cttcgctgta atatccaaaa tcaccagaat

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<210> 186

<211> 40

20<212> DNA

<213> Artificial Sequence

<220>

<223> An oligonucleotide

25

<400> 186

gcaaccagcc gtcgctgctg atggcctcct tggtagcttc

40

<210> 187

30<211> 40

<212> DNA

<213> Artificial Sequence

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35<223> An oligonucleotide

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gacgttattg acataaccct tgctcaccat agggcctttg

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40<210> 188

<211> 40

<212> DNA

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atacacagct cgcccacttg gttagggccc aaagccttac

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<210> 189

<211> 40

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<213> Artificial Sequence

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40

<210> 190

<211> 40

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25<213> Artificial Sequence

<220>

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gacacggccc aaagagcgc tcttaaactc atcgcggaga

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<210> 191

<211> 37

35<212> DNA

<213> Artificial Sequence

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<223> An oligonucleotide

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&lt;210&gt; 192

5&lt;211&gt; 43

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

10&lt;223&gt; An oligonucleotide

&lt;400&gt; 192

agccacaacg aatccctgga agattcaagc gtttggcggc cac

43

15&lt;210&gt; 193

&lt;211&gt; 40

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

20&lt;220&gt;

&lt;223&gt; An oligonucleotide

&lt;400&gt; 193

ttcagcgacc tccttagcca gtggagcggc accgcaacac

40

25

&lt;210&gt; 194

&lt;211&gt; 40

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

30

&lt;220&gt;

&lt;223&gt; An oligonucleotide

&lt;400&gt; 194

35aattcagca gtgaagacaa gtcgtacttg tccacgagtg

40

&lt;210&gt; 195

&lt;211&gt; 40

&lt;212&gt; DNA

40&lt;213&gt; Artificial Sequence

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<400> 195

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40

<210> 196

<211> 40

<212> DNA

10<213> Artificial Sequence

<220>

<223> An oligonucleotide

15<400> 196

gacactgcgg acttcataat cttggatggc tttcaagaaa

40

<210> 197

<211> 40

20<212> DNA

<213> Artificial Sequence

<220>

<223> An oligonucleotide

25

<400> 197

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40

<210> 198

30<211> 40

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cgaccataaa gtaaccctaaa gtaatgatgaa agccgaaagc

40

40<210> 199

<211> 40

<212> DNA

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5<220>

<223> An oligonucleotide

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atggaagaaa ggcaagtaga ccaagacggt gacaccagga

40

10

<210> 200

<211> 40

<212> DNA

<213> Artificial Sequence

15

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20atcagctgag tgccgtagcg tggatcgaga gcatggatca

40

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25<213> Artificial Sequence

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30<400> 201

gacgcacgca aatgttttga tgggtctgca tgactccctt

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<210> 202

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&lt;210&gt; 203

5&lt;211&gt; 40

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

10&lt;223&gt; An oligonucleotide

&lt;400&gt; 203

acttggtcca cagggtcgaa gtggagtggg ttaaagtttg

40

15&lt;210&gt; 204

&lt;211&gt; 40

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

20&lt;220&gt;

&lt;223&gt; An oligonucleotide

&lt;400&gt; 204

cgatgttgcc gtctgaatag cgagagatga aattaggcaa

40

25

&lt;210&gt; 205

&lt;211&gt; 40

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

30

&lt;220&gt;

&lt;223&gt; An oligonucleotide

&lt;400&gt; 205

35agattcgcaa ccgtgaatat tctccacagt gtccaagatg

40

&lt;210&gt; 206

&lt;211&gt; 40

&lt;212&gt; DNA

40&lt;213&gt; Artificial Sequence



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<223> An oligonucleotide

~~<400> 206~~

Satgatagcgt taataaagtt ggtgcggctt tggacttcca

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<210> 207

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<212> DNA

10<213> Artificial Sequence

<220>

<223> An oligonucleotide

15<400> 207

ggactttggt cagaatattc ttagtggtga agacaatctg

40

<210> 208

<211> 40

20<212> DNA

<213> Artificial Sequence

<220>

<223> An oligonucleotide

25

<400> 208

tggcttagag ataccatga ctttacacag ttcgtcggga

40

<210> 209

30<211> 40

<212> DNA

<213> Artificial Sequence

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35<223> An oligonucleotide

<400> 209

atgtagctct cgttgactgg agccacgatc ataccgatat

40

40<210> 210

<211> 40

<212> DNA

<213> Artificial Sequence

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<223> An oligonucleotide

<400> 210

accatgcggc gatgactgga atgaagaaac ggggtattgtt

40

10

<210> 211

<211> 40

<212> DNA

<213> Artificial Sequence

15

<220>

<223> An oligonucleotide

<400> 211

20tttcagcacag atactaacga cgtcggttcac cttgtagcca

40

<210> 212

<211> 40

<212> DNA

25<213> Artificial Sequence

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<223> An oligonucleotide

30<400> 212

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40

<210> 213

<211> 40

35<212> DNA

<213> Artificial Sequence

<220>

<223> An oligonucleotide

40

&lt;400&gt; 213

aaaactcctt gtagctcaaa gattcatcgc cgaccacatc

40

&lt;210&gt; 214

5&lt;211&gt; 40

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

10&lt;223&gt; An oligonucleotide

&lt;400&gt; 214

gaccaaggct tgaggcaaat gagagtgcct gcggagagca

40

15&lt;210&gt; 215

&lt;211&gt; 40

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

20&lt;220&gt;

&lt;223&gt; An oligonucleotide

&lt;400&gt; 215

cgaaacagca tttcgccggc agtcaaatac tocaaaggat

40

25

&lt;210&gt; 216

&lt;211&gt; 40

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

30

&lt;220&gt;

&lt;223&gt; An oligonucleotide

&lt;400&gt; 216

35ggagaggctc agggccatag atgacatttt tctcacgctt

40

&lt;210&gt; 217

&lt;211&gt; 40

&lt;212&gt; DNA

40&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; An oligonucleotide

&lt;400&gt; 217

5catcatggga tcctgtttcc tgtgtgaaat tggtatccgc

40

&lt;210&gt; 218

&lt;211&gt; 542

&lt;212&gt; PRT

10&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Sequence of a synthetic luciferase

15&lt;400&gt; 218

|       |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met   | Met | Lys | Arg | Glu | Lys | Asn | Val | Ile | Tyr | Gly | Pro | Glu | Pro | Leu | His |
| 1     |     |     |     |     | 5   |     |     |     |     | 10  |     |     |     | 15  |     |
| Pro   | Leu | Glu | Asp | Leu | Thr | Ala | Gly | Glu | Met | Leu | Phe | Arg | Ala | Leu | Arg |
|       |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |
| 20Lys | His | Ser | His | Leu | Pro | Gln | Ala | Leu | Val | Asp | Val | Val | Gly | Asp | Glu |
|       |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |
| Ser   | Leu | Ser | Tyr | Lys | Glu | Phe | Phe | Glu | Ala | Thr | Val | Leu | Leu | Ala | Gln |
|       | 50  |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |
| Ser   | Leu | His | Asn | Cys | Gly | Tyr | Lys | Met | Asn | Asp | Val | Val | Ser | Ile | Cys |
| 2565  |     |     |     | 70  |     |     |     |     | 75  |     |     |     |     | 80  |     |
| Ala   | Glu | Asn | Asn | Thr | Arg | Phe | Phe | Ile | Pro | Val | Ile | Ala | Ala | Trp | Tyr |
|       |     |     | 85  |     |     |     |     | 90  |     |     |     | 95  |     |     |     |
| Ile   | Gly | Met | Ile | Val | Ala | Pro | Val | Asn | Glu | Ser | Tyr | Ile | Pro | Asp | Glu |
|       |     | 100 |     |     |     |     | 105 |     |     |     |     | 110 |     |     |     |
| 30Leu | Cys | Lys | Val | Met | Gly | Ile | Ser | Lys | Pro | Gln | Ile | Val | Phe | Thr | Thr |
|       |     | 115 |     |     |     |     | 120 |     |     |     |     | 125 |     |     |     |
| Lys   | Asn | Ile | Leu | Asn | Lys | Val | Leu | Glu | Val | Gln | Ser | Arg | Thr | Asn | Phe |
|       | 130 |     |     |     |     | 135 |     |     |     |     | 140 |     |     |     |     |
| Ile   | Lys | Arg | Ile | Ile | Ile | Leu | Asp | Thr | Val | Glu | Asn | Ile | His | Gly | Cys |
| 35145 |     |     |     | 150 |     |     |     |     | 155 |     |     |     | 160 |     |     |
| Glu   | Ser | Leu | Pro | Asn | Phe | Ile | Ser | Arg | Tyr | Ser | Asp | Gly | Asn | Ile | Ala |
|       |     |     | 165 |     |     |     |     | 170 |     |     |     | 175 |     |     |     |
| Asn   | Phe | Lys | Pro | Leu | His | Phe | Asp | Pro | Val | Glu | Gln | Val | Ala | Ala | Ile |
|       |     | 180 |     |     |     |     |     | 185 |     |     |     | 190 |     |     |     |
| 40Leu | Cys | Ser | Ser | Gly | Thr | Thr | Gly | Leu | Pro | Lys | Gly | Val | Met | Gln | Thr |

|  |     |     |
|--|-----|-----|
| 195  | 200 | 205 |
| His Gln Asn Ile Cys Val Arg Leu Ile His Ala Leu Asp Pro Arg Tyr            |     |     |
| 210  | 215 | 220 |
| <del>Gly Thr Gln Leu Ile Pro Gly Val Thr Val Leu Val Tyr Leu Pro Phe</del> |     |     |
| 5225   | 230 | 235 |
| Phe His Ala Phe Gly Phe His Ile Thr Leu Gly Tyr Phe Met Val Gly            |     |     |
|  | 245 | 250 |
| Leu Arg Val Ile Met Phe Arg Arg Phe Asp Gln Glu Ala Phe Leu Lys            |     |     |
|  | 260 | 265 |
| 10Ala Ile Gln Asp Tyr Glu Val Arg Ser Val Ile Asn Val Pro Ser Val          |     |     |
|  | 275 | 280 |
| Ile Leu Phe Leu Ser Lys Ser Pro Leu Val Asp Lys Tyr Asp Leu Ser            |     |     |
|  | 290 | 295 |
| Ser Leu Arg Glu Leu Cys Cys Gly Ala Ala Pro Leu Ala Lys Glu Val            |     |     |
| 15305  | 310 | 315 |
| Ala Glu Val Ala Ala Lys Arg Leu Asn Leu Pro Gly Ile Arg Cys Gly            |     |     |
|  | 325 | 330 |
| Phe Gly Leu Thr Glu Ser Thr Ser Ala Ile Ile Gln Ser Leu Arg Asp            |     |     |
|  | 340 | 345 |
| 20Glu Phe Lys Ser Gly Ser Leu Gly Arg Val Thr Pro Leu Met Ala Ala          |     |     |
|  | 355 | 360 |
| Lys Ile Ala Asp Arg Glu Thr Gly Lys Ala Leu Gly Pro Asn Gln Val            |     |     |
|  | 370 | 375 |
| Gly Glu Leu Cys Ile Lys Gly Pro Met Val Ser Lys Gly Tyr Val Asn            |     |     |
| 25385  | 390 | 395 |
| Asn Val Glu Ala Thr Lys Glu Ala Ile Asp Asp Asp Gly Trp Leu His            |     |     |
|  | 405 | 410 |
| Ser Gly Asp Phe Gly Tyr Tyr Asp Glu Asp Glu His Phe Tyr Val Val            |     |     |
|  | 420 | 425 |
| 30Asp Arg Tyr Lys Glu Leu Ile Lys Tyr Lys Gly Ser Gln Val Ala Pro          |     |     |
|  | 435 | 440 |
| Ala Glu Leu Glu Glu Ile Leu Leu Lys Asn Pro Cys Ile Arg Asp Val            |     |     |
|  | 450 | 455 |
| Ala Val Val Gly Ile Pro Asp Leu Glu Ala Gly Glu Leu Pro Ser Ala            |     |     |
| 35465  | 470 | 475 |
| Phe Val Val Lys Gln Pro Gly Lys Glu Ile Thr Ala Lys Glu Val Tyr            |     |     |
|  | 485 | 490 |
| Asp Tyr Leu Ala Glu Arg Val Ser His Thr Lys Tyr Leu Arg Gly Gly            |     |     |
|  | 500 | 505 |
| 40Val Arg Phe Val Asp Ser Ile Pro Arg Asn Val Thr Gly Lys Ile Thr          |     |     |

515 520 525  
 Arg Lys Glu Leu Leu Lys Gln Leu Leu Glu Lys Ala Gly Gly  
 530 535 540

5<210> 219

<211> 542

<212> PRT

<213> Artificial Sequence

10<220>

<223> Sequence of a synthetic luciferase

<400> 219

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 15 1 5 10 15  
 Pro Leu Glu Asp Leu Thr Ala Gly Glu Met Leu Phe Arg Ala Leu Arg  
 20 25 30  
 Lys His Ser His Leu Pro Gln Ala Leu Val Asp Val Val Gly Asp Glu  
 35 40 45  
 20Ser Leu Ser Tyr Lys Glu Phe Phe Glu Ala Thr Val Leu Leu Ala Gln  
 50 55 60  
 Ser Leu His Asn Cys Gly Tyr Lys Met Asn Asp Val Val Ser Ile Cys  
 65 70 75 80  
 Ala Glu Asn Asn Thr Arg Phe Phe Ile Pro Val Ile Ala Ala Trp Tyr  
 25 85 90 95  
 Ile Gly Met Ile Val Ala Pro Val Asn Glu Ser Tyr Ile Pro Asp Glu  
 100 105 110  
 Leu Cys Lys Val Met Gly Ile Ser Lys Pro Gln Ile Val Phe Thr Thr  
 115 120 125  
 30Lys Asn Ile Leu Asn Lys Val Leu Glu Val Gln Ser Arg Thr Asn Phe  
 130 135 140  
 Ile Lys Arg Ile Ile Ile Leu Asp Thr Val Glu Asn Ile His Gly Cys  
 145 150 155 160  
 Glu Ser Leu Pro Asn Phe Ile Ser Arg Tyr Ser Asp Gly Asn Ile Ala  
 35 165 170 175  
 Asn Phe Lys Pro Leu His Phe Asp Pro Val Glu Gln Val Ala Ala Ile  
 180 185 190  
 Leu Cys Ser Ser Gly Thr Thr Gly Leu Pro Lys Gly Val Met Gln Thr  
 195 200 205  
 40His Gln Asn Ile Cys Val Arg Leu Ile His Ala Leu Asp Pro Arg Tyr

210                      215                      220  
 Gly Thr Gln Leu Ile Pro Gly Val Thr Val Leu Val Tyr Leu Pro Phe  
 225                      230                      235                      240  
 Phe His Ala Phe Gly Phe His Ile Thr Leu Gly Tyr Phe Met Val Gly  
 5                      245                      250                      255  
 Leu Arg Val Ile Met Phe Arg Arg Phe Asp Gln Glu Ala Phe Leu Lys  
 260                      265                      270  
 Ala Ile Gln Asp Tyr Glu Val Arg Ser Val Ile Asn Val Pro Ser Val  
 275                      280                      285  
 10 Ile Leu Phe Leu Ser Lys Ser Pro Leu Val Asp Lys Tyr Asp Leu Ser  
 290                      295                      300  
 Ser Leu Arg Glu Leu Cys Cys Gly Ala Ala Pro Leu Ala Lys Glu Val  
 305                      310                      315                      320  
 Ala Glu Val Ala Ala Lys Arg Leu Asn Leu Pro Gly Ile Arg Cys Gly  
 15                      325                      330                      335  
 Phe Gly Leu Thr Glu Ser Thr Ser Ala Ile Ile Gln Ser Leu Arg Asp  
 340                      345                      350  
 Glu Phe Lys Ser Gly Ser Leu Gly Arg Val Thr Pro Leu Met Ala Ala  
 355                      360                      365  
 20 Lys Ile Ala Asp Arg Glu Thr Gly Lys Ala Leu Gly Pro Asn Gln Val  
 370                      375                      380  
 Gly Glu Leu Cys Ile Lys Gly Pro Met Val Ser Lys Gly Tyr Val Asn  
 385                      390                      395                      400  
 Asn Val Glu Ala Thr Lys Glu Ala Ile Asp Asp Asp Gly Trp Leu His  
 25                      405                      410                      415  
 Ser Gly Asp Phe Gly Tyr Tyr Asp Glu Asp Glu His Phe Tyr Val Val  
 420                      425                      430  
 Asp Arg Tyr Lys Glu Leu Ile Lys Tyr Lys Gly Ser Gln Val Ala Pro  
 435                      440                      445  
 30 Ala Glu Leu Glu Glu Ile Leu Leu Lys Asn Pro Cys Ile Arg Asp Val  
 450                      455                      460  
 Ala Val Val Gly Ile Pro Asp Leu Glu Ala Gly Glu Leu Pro Ser Ala  
 465                      470                      475                      480  
 Phe Val Val Lys Gln Pro Gly Lys Glu Ile Thr Ala Lys Glu Val Tyr  
 35                      485                      490                      495  
 Asp Tyr Leu Ala Glu Arg Val Ser His Thr Lys Tyr Leu Arg Gly Gly  
 500                      505                      510  
 Val Arg Phe Val Asp Ser Ile Pro Arg Asn Val Thr Gly Lys Ile Thr  
 515                      520                      525  
 40 Arg Lys Glu Leu Leu Lys Gln Leu Leu Glu Lys Ala Gly Gly

530

535

540

&lt;210&gt; 220

&lt;211&gt; 542

5&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Sequence of a synthetic luciferase

10

&lt;400&gt; 220

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Met Met Lys Arg Glu Lys Asn Val Ile Tyr Gly Pro Glu Pro Leu His
  1             5             10             15
Pro Leu Glu Asp Leu Thr Ala Gly Glu Met Leu Phe Arg Ala Leu Arg
15             20             25             30
Lys His Ser Tyr Leu Pro Gln Ala Leu Val Asp Val Val Gly Asp Glu
      35             40             45
Ser Leu Ser Tyr Lys Glu Phe Phe Glu Ala Thr Val Leu Leu Ala Gln
      50             55             60
20Ser Leu His Asn Cys Gly Tyr Lys Met Asn Asp Val Val Ser Ile Cys
   65             70             75             80
Ala Glu Asn Asn Thr Arg Phe Phe Ile Pro Val Ile Ala Ala Trp Tyr
      85             90             95
Ile Gly Met Ile Val Ala Pro Val Asn Glu Ser Tyr Ile Pro Asp Glu
25             100            105            110
Leu Cys Lys Val Met Gly Ile Ser Lys Pro Gln Ile Val Phe Thr Thr
      115            120            125
Lys Asn Ile Leu Asn Lys Val Leu Glu Val Gln Ser Arg Thr Asn Phe
      130            135            140
30Ile Lys Arg Ile Ile Ile Leu Asp Thr Val Glu Asn Ile His Gly Cys
   145            150            155            160
Glu Ser Leu Pro Asn Phe Ile Ser Arg Tyr Ser Asp Gly Asn Ile Ala
      165            170            175
Asn Phe Lys Pro Leu His Phe Asp Pro Val Glu Gln Val Ala Ala Ile
35             180             185             190
Leu Cys Ser Ser Gly Thr Thr Gly Leu Pro Lys Gly Val Met Gln Thr
      195            200            205
His Gln Asn Ile Cys Val Arg Leu Ile His Ala Leu Asp Pro Arg Tyr
      210            215            220
40Gly Thr Gln Leu Ile Pro Gly Val Thr Val Leu Val Tyr Leu Pro Phe

```



99

|   |   |     |     |     |     |     |
|---|---|-----|-----|-----|-----|-----|
| 225   |   | 230 |     | 235 |     | 240 |
| Phe His Ala Phe Gly   | Phe His Ile Thr Leu Gly Tyr Phe Met Val Gly |     |     |     |     |     |
|   | 245   |     | 250 |     | 255 |     |
| Leu Arg Val Ile Met Phe Arg Arg Phe Asp Gln Glu Ala Phe Leu Lys   |   |     |     |     |     |     |
| 5   | 260   |     | 265 |     | 270 |     |
| Ala Ile Gln Asp Tyr Glu Val Arg Ser Val Ile Asn Val Pro Ser Val   |   |     |     |     |     |     |
|   | 275   |     | 280 |     | 285 |     |
| Ile Leu Phe Leu Ser Lys Ser Pro Leu Val Asp Lys Tyr Asp Leu Ser   |   |     |     |     |     |     |
| 290   |   | 295 |     | 300 |     |     |
| 10Ser Leu Arg Glu Leu Cys Cys Gly Ala Ala Pro Leu Ala Lys Glu Val |   |     |     |     |     |     |
| 305   |   | 310 |     | 315 |     | 320 |
| Ala Glu Val Ala Ala Lys Arg Leu Asn Leu Pro Gly Ile Arg Cys Gly   |   |     |     |     |     |     |
|   | 325   |     | 330 |     | 335 |     |
| Phe Gly Leu Thr Glu Ser Thr Ser Ala Ile Ile Gln Ser Leu Arg Asp   |   |     |     |     |     |     |
| 15  | 340   |     | 345 |     | 350 |     |
| Glu Phe Lys Ser Gly Ser Leu Gly Arg Val Thr Pro Leu Met Ala Ala   |   |     |     |     |     |     |
|   | 355   |     | 360 |     | 365 |     |
| Lys Ile Ala Asp Arg Glu Thr Gly Lys Ala Leu Gly Pro Asn Gln Val   |   |     |     |     |     |     |
| 370   |   | 375 |     | 380 |     |     |
| 20Gly Glu Leu Cys Ile Lys Gly Pro Met Val Ser Lys Gly Tyr Val Asn |   |     |     |     |     |     |
| 385   |   | 390 |     | 395 |     | 400 |
| Asn Val Glu Ala Thr Lys Glu Ala Ile Asp Asp Asp Gly Trp Leu His   |   |     |     |     |     |     |
|   | 405   |     | 410 |     | 415 |     |
| Ser Gly Asp Phe Gly Tyr Tyr Asp Glu Asp Glu His Phe Tyr Val Val   |   |     |     |     |     |     |
| 25  | 420   |     | 425 |     | 430 |     |
| Asp Arg Tyr Lys Glu Leu Ile Lys Tyr Lys Gly Ser Gln Val Ala Pro   |   |     |     |     |     |     |
|   | 435   |     | 440 |     | 445 |     |
| Ala Glu Leu Glu Glu Ile Leu Leu Lys Asn Pro Cys Ile Arg Asp Val   |   |     |     |     |     |     |
| 450   |   | 455 |     | 460 |     |     |
| 30Ala Val Val Gly Ile Pro Asp Leu Glu Ala Gly Glu Leu Pro Ser Ala |   |     |     |     |     |     |
| 465   |   | 470 |     | 475 |     | 480 |
| Phe Val Val Lys Gln Pro Gly Lys Glu Ile Thr Ala Lys Glu Val Tyr   |   |     |     |     |     |     |
|   | 485   |     | 490 |     | 495 |     |
| Asp Tyr Leu Ala Glu Arg Val Ser His Thr Lys Tyr Leu Arg Gly Gly   |   |     |     |     |     |     |
| 35  | 500   |     | 505 |     | 510 |     |
| Val Arg Phe Val Asp Ser Ile Pro Arg Asn Val Thr Gly Lys Ile Thr   |   |     |     |     |     |     |
|   | 515   |     | 520 |     | 525 |     |
| Arg Lys Glu Leu Leu Lys Gln Leu Leu Glu Lys Ala Gly Gly           |   |     |     |     |     |     |
| 530   |   | 535 |     | 540 |     |     |

100

&lt;210&gt; 221

&lt;211&gt; 542

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

5

&lt;220&gt;

&lt;223&gt; Sequence of a synthetic luciferase

&lt;400&gt; 221

```

10Met Met Lys Arg Glu Lys Asn Val Ile Tyr Gly Pro Glu Pro Leu His
   1             5             10             15
   Pro Leu Glu Asp Leu Thr Ala Gly Glu Met Leu Phe Arg Ala Leu Arg
           20             25             30
   Lys His Ser His Leu Pro Gln Ala Leu Val Asp Val Val Gly Asp Glu
15           35             40             45
   Ser Leu Ser Tyr Lys Glu Phe Phe Glu Ala Thr Val Leu Leu Ala Gln
           50             55             60
   Ser Leu His Asn Cys Gly Tyr Lys Met Asn Asp Val Val Ser Ile Cys
   65           70             75             80
20Ala Glu Asn Asn Thr Arg Phe Phe Ile Pro Val Ile Ala Ala Trp Tyr
           85             90             95
   Ile Gly Met Ile Val Ala Pro Val Asn Glu Ser Tyr Ile Pro Asp Glu
           100            105            110
   Leu Cys Lys Val Met Gly Ile Ser Lys Pro Gln Ile Val Phe Thr Thr
25           115            120            125
   Lys Asn Ile Leu Asn Lys Val Leu Glu Val Gln Ser Arg Thr Asn Phe
           130            135            140
   Ile Lys Arg Ile Ile Ile Leu Asp Thr Val Glu Asn Ile His Gly Cys
   145           150            155            160
30Glu Ser Leu Pro Asn Phe Ile Ser Arg Tyr Ser Asp Gly Asn Ile Ala
           165            170            175
   Asn Phe Lys Pro Leu His Phe Asp Pro Val Glu Gln Val Ala Ala Ile
           180            185            190
   Leu Cys Ser Ser Gly Thr Thr Gly Leu Pro Lys Gly Val Met Gln Thr
35           195            200            205
   His Gln Asn Ile Cys Val Arg Leu Ile His Ala Leu Asp Pro Arg Tyr
           210            215            220
   Gly Thr Gln Leu Ile Pro Gly Val Thr Val Leu Val Tyr Leu Pro Phe
   225           230            235            240
40Phe His Ala Phe Gly Phe His Ile Thr Leu Gly Tyr Phe Met Val Gly

```

101

|    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |  |  |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|--|--|
|    |     | 245 |     | 250 |     | 255 |     |     |     |     |     |     |     |     |     |     |  |  |  |
|    | Leu | Arg | Val | Ile | Met | Phe | Arg | Arg | Phe | Asp | Gln | Glu | Ala | Phe | Leu | Lys |  |  |  |
|    |     |     | 260 |     |     |     |     |     | 265 |     |     |     |     | 270 |     |     |  |  |  |
|    | Ala | Ile | Gln | Asp | Tyr | Glu | Val | Arg | Ser | Val | Ile | Asn | Val | Pro | Ser | Val |  |  |  |
| 5  |     |     | 275 |     |     |     |     |     | 280 |     |     |     |     | 285 |     |     |  |  |  |
|    | Ile | Leu | Phe | Leu | Ser | Lys | Ser | Pro | Leu | Val | Asp | Lys | Tyr | Asp | Leu | Ser |  |  |  |
|    |     |     | 290 |     |     |     |     | 295 |     |     |     |     | 300 |     |     |     |  |  |  |
|    | Ser | Leu | Arg | Glu | Leu | Cys | Cys | Gly | Ala | Ala | Pro | Leu | Ala | Lys | Glu | Val |  |  |  |
|    | 305 |     |     |     |     | 310 |     |     |     |     | 315 |     |     |     | 320 |     |  |  |  |
| 10 | Ala | Glu | Val | Ala | Ala | Lys | Arg | Leu | Asn | Leu | Pro | Gly | Ile | Arg | Cys | Gly |  |  |  |
|    |     |     |     |     |     | 325 |     |     |     | 330 |     |     |     |     | 335 |     |  |  |  |
|    | Phe | Gly | Leu | Thr | Glu | Ser | Thr | Ser | Ala | Ile | Ile | Gln | Ser | Leu | Arg | Asp |  |  |  |
|    |     |     | 340 |     |     |     |     |     | 345 |     |     |     |     | 350 |     |     |  |  |  |
|    | Glu | Phe | Lys | Ser | Gly | Ser | Leu | Gly | Arg | Val | Thr | Pro | Leu | Met | Ala | Ala |  |  |  |
| 15 |     |     | 355 |     |     |     |     |     | 360 |     |     |     |     | 365 |     |     |  |  |  |
|    | Lys | Ile | Ala | Asp | Arg | Glu | Thr | Gly | Lys | Ala | Leu | Gly | Pro | Asn | Gln | Val |  |  |  |
|    |     |     | 370 |     |     |     |     | 375 |     |     |     |     | 380 |     |     |     |  |  |  |
|    | Gly | Glu | Leu | Cys | Ile | Lys | Gly | Pro | Met | Val | Ser | Lys | Gly | Tyr | Val | Asn |  |  |  |
|    | 385 |     |     |     |     | 390 |     |     |     |     | 395 |     |     |     | 400 |     |  |  |  |
| 20 | Asn | Val | Glu | Ala | Thr | Lys | Glu | Ala | Ile | Asp | Asp | Asp | Gly | Trp | Leu | His |  |  |  |
|    |     |     |     |     |     | 405 |     |     |     | 410 |     |     |     |     | 415 |     |  |  |  |
|    | Ser | Gly | Asp | Phe | Gly | Tyr | Tyr | Asp | Glu | Asp | Glu | His | Phe | Tyr | Val | Val |  |  |  |
|    |     |     | 420 |     |     |     |     |     | 425 |     |     |     |     | 430 |     |     |  |  |  |
|    | Asp | Arg | Tyr | Lys | Glu | Leu | Ile | Lys | Tyr | Lys | Gly | Ser | Gln | Val | Ala | Pro |  |  |  |
| 25 |     |     | 435 |     |     |     |     |     | 440 |     |     |     |     | 445 |     |     |  |  |  |
|    | Ala | Glu | Leu | Glu | Glu | Ile | Leu | Leu | Lys | Asn | Pro | Cys | Ile | Arg | Asp | Val |  |  |  |
|    |     |     | 450 |     |     |     |     |     | 455 |     |     |     |     | 460 |     |     |  |  |  |
|    | Ala | Val | Val | Gly | Ile | Pro | Asp | Leu | Glu | Ala | Gly | Glu | Leu | Pro | Ser | Ala |  |  |  |
|    | 465 |     |     |     |     |     | 470 |     |     |     | 475 |     |     |     | 480 |     |  |  |  |
| 30 | Phe | Val | Val | Lys | Gln | Pro | Gly | Lys | Glu | Ile | Thr | Ala | Lys | Glu | Val | Tyr |  |  |  |
|    |     |     |     |     |     | 485 |     |     |     |     | 490 |     |     |     | 495 |     |  |  |  |
|    | Asp | Tyr | Leu | Ala | Glu | Arg | Val | Ser | His | Thr | Lys | Tyr | Leu | Arg | Gly | Gly |  |  |  |
|    |     |     | 500 |     |     |     |     |     | 505 |     |     |     |     | 510 |     |     |  |  |  |
|    | Val | Arg | Phe | Val | Asp | Ser | Ile | Pro | Arg | Asn | Val | Thr | Gly | Lys | Ile | Thr |  |  |  |
| 35 |     |     | 515 |     |     |     |     |     | 520 |     |     |     |     | 525 |     |     |  |  |  |
|    | Arg | Lys | Glu | Leu | Leu | Lys | Gln | Leu | Leu | Glu | Lys | Ala | Gly | Gly |     |     |  |  |  |
|    |     |     | 530 |     |     |     |     | 535 |     |     |     |     |     | 540 |     |     |  |  |  |

&lt;210&gt; 222

40&lt;211&gt; 542

102

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

5&lt;223&gt; Sequence of a synthetic luciferase

&lt;400&gt; 222

```

Met Met Lys Arg Glu Lys Asn Val Ile Tyr Gly Pro Glu Pro Leu His
  1             5             10             15
10Pro Leu Glu Asp Leu Thr Ala Gly Glu Met Leu Phe Arg Ala Leu Arg
      20             25             30
Lys His Ser His Leu Pro Gln Ala Leu Val Asp Val Val Gly Asp Glu
      35             40             45
Ser Leu Ser Tyr Lys Glu Phe Phe Glu Ala Thr Val Leu Leu Ala Gln
15    50             55             60
Ser Leu His Asn Cys Gly Tyr Lys Met Asn Asp Val Val Ser Ile Cys
65             70             75             80
Ala Glu Asn Asn Thr Arg Phe Phe Ile Pro Val Ile Ala Ala Trp Tyr
      85             90             95
20Ile Gly Met Ile Val Ala Pro Val Asn Glu Ser Tyr Ile Pro Asp Glu
      100            105            110
Leu Cys Lys Val Met Gly Ile Ser Lys Pro Gln Ile Val Phe Thr Thr
      115            120            125
Lys Asn Ile Leu Asn Lys Val Leu Glu Val Gln Ser Arg Thr Asn Phe
25    130            135            140
Ile Lys Arg Ile Ile Ile Leu Asp Thr Val Glu Asn Ile His Gly Cys
145            150            155            160
Glu Ser Leu Pro Asn Phe Ile Ser Arg Tyr Ser Asp Gly Asn Ile Ala
      165            170            175
30Asn Phe Lys Pro Leu His Phe Asp Pro Val Glu Gln Val Ala Ala Ile
      180            185            190
Leu Cys Ser Ser Gly Thr Thr Gly Leu Pro Lys Gly Val Met Gln Thr
      195            200            205
His Gln Asn Ile Cys Val Arg Leu Ile His Ala Leu Asp Pro Arg Tyr
35    210            215            220
Gly Thr Gln Leu Ile Pro Gly Val Thr Val Leu Val Tyr Leu Pro Phe
225            230            235            240
Phe His Ala Phe Gly Phe His Ile Thr Leu Gly Tyr Phe Met Val Gly
      245            250            255
40Leu Arg Val Ile Met Phe Arg Arg Phe Asp Gln Glu Ala Phe Leu Lys

```

103

|       |   |     |     |     |     |
|-------|---|-----|-----|-----|-----|
|       | 260   |     | 265 |     | 270 |
|       | Ala Ile Gln Asp Tyr Glu Val Arg Ser Val Ile Asn Val Pro Ser Val |     |     |     |     |
|       | 275   |     | 280 |     | 285 |
| ----- | Ile Leu Phe Leu Ser Lys Ser Pro Leu Val Asp Lys Tyr Asp Leu Ser |     |     |     |     |
| 5     | 290   |     | 295 |     | 300 |
|       | Ser Leu Arg Glu Leu Cys Cys Gly Ala Ala Pro Leu Ala Lys Glu Val |     |     |     |     |
|       | 305   |     | 310 |     | 315 |
|       | Ala Glu Val Ala Ala Lys Arg Leu Asn Leu Pro Gly Ile Arg Cys Gly |     |     |     |     |
|       |   | 325 |     | 330 | 335 |
| 10    | Phe Gly Leu Thr Glu Ser Thr Ser Ala Ile Ile Gln Ser Leu Gly Asp |     |     |     |     |
|       |   | 340 |     | 345 | 350 |
|       | Glu Phe Lys Ser Gly Ser Leu Gly Arg Val Thr Pro Leu Met Ala Ala |     |     |     |     |
|       |   | 355 |     | 360 | 365 |
|       | Lys Ile Ala Asp Arg Glu Thr Gly Lys Ala Leu Gly Pro Asn Gln Val |     |     |     |     |
| 15    | 370   |     | 375 |     | 380 |
|       | Gly Glu Leu Cys Ile Lys Gly Pro Met Val Ser Lys Gly Tyr Val Asn |     |     |     |     |
|       |   | 385 |     | 390 | 395 |
|       | Asn Val Glu Ala Thr Lys Glu Ala Ile Asp Asp Asp Gly Trp Leu His |     |     |     |     |
|       |   | 405 |     | 410 | 415 |
| 20    | Ser Gly Asp Phe Gly Tyr Tyr Asp Glu Asp Glu His Phe Tyr Val Val |     |     |     |     |
|       |   | 420 |     | 425 | 430 |
|       | Asp Arg Tyr Lys Glu Leu Ile Lys Tyr Lys Gly Ser Gln Val Ala Pro |     |     |     |     |
|       |   | 435 |     | 440 | 445 |
|       | Ala Glu Leu Glu Glu Ile Leu Leu Lys Asn Pro Cys Ile Arg Asp Val |     |     |     |     |
| 25    | 450   |     | 455 |     | 460 |
|       | Ala Val Val Gly Ile Pro Asp Leu Glu Ala Gly Glu Leu Pro Ser Ala |     |     |     |     |
|       |   | 465 |     | 470 | 475 |
|       | Phe Val Val Lys Gln Pro Gly Lys Glu Ile Thr Ala Lys Glu Val Tyr |     |     |     |     |
|       |   | 485 |     | 490 | 495 |
| 30    | Asp Tyr Leu Ala Glu Arg Val Ser His Thr Lys Tyr Leu Arg Gly Gly |     |     |     |     |
|       |   | 500 |     | 505 | 510 |
|       | Val Arg Phe Val Asp Ser Ile Pro Arg Asn Val Thr Gly Lys Ile Thr |     |     |     |     |
|       |   | 515 |     | 520 | 525 |
|       | Arg Lys Glu Leu Leu Lys Gln Leu Leu Glu Lys Ala Gly Gly         |     |     |     |     |
| 35    | 530   |     | 535 |     | 540 |

&lt;210&gt; 223

&lt;211&gt; 542

&lt;212&gt; PRT

40&lt;213&gt; Artificial Sequence

104

&lt;220&gt;

&lt;223&gt; Sequence of a synthetic luciferase

&lt;400&gt; 223

```

5Met Ile Lys Arg Glu Lys Asn Val Ile Tyr Gly Pro Glu Pro Leu His
  1             5             10             15
Pro Leu Glu Asp Leu Thr Ala Gly Glu Met Leu Phe Arg Ala Leu Arg
      20             25             30
Lys His Ser His Leu Pro Gln Ala Leu Val Asp Val Val Gly Asp Glu
10      35             40             45
Ser Leu Ser Tyr Lys Glu Phe Phe Glu Ala Thr Val Leu Leu Ala Gln
      50             55             60
Ser Leu His Asn Cys Gly Tyr Lys Met Asn Asp Val Val Ser Ile Cys
65      70             75             80
15Ala Glu Asn Asn Thr Arg Phe Phe Ile Pro Val Ile Ala Ala Trp Tyr
      85             90             95
Ile Gly Met Ile Val Ala Pro Val Asn Glu Ser Tyr Ile Pro Asp Glu
      100            105            110
Leu Cys Lys Val Met Gly Ile Ser Lys Pro Gln Ile Val Phe Thr Thr
20      115            120            125
Lys Asn Ile Leu Asn Lys Val Leu Glu Val Gln Ser Arg Thr Asn Phe
      130            135            140
Ile Lys Arg Ile Ile Ile Leu Asp Thr Val Glu Asn Ile His Gly Cys
145      150            155            160
25Glu Ser Leu Pro Asn Phe Ile Ser Arg Tyr Ser Asp Gly Asn Ile Ala
      165            170            175
Asn Phe Lys Pro Leu His Phe Asp Pro Val Glu Gln Val Ala Ala Ile
      180            185            190
Leu Cys Ser Ser Gly Thr Thr Gly Leu Pro Lys Gly Val Met Gln Thr
30      195            200            205
His Gln Asn Ile Cys Val Arg Leu Ile His Ala Leu Asp Pro Arg Tyr
      210            215            220
Gly Thr Gln Leu Ile Pro Gly Val Thr Val Leu Val Tyr Leu Pro Phe
225      230            235            240
35Phe His Ala Phe Gly Phe His Ile Thr Leu Gly Tyr Phe Met Val Gly
      245            250            255
Leu Arg Val Ile Met Phe Arg Arg Phe Asp Gln Glu Ala Phe Leu Lys
      260            265            270
Ala Ile Gln Asp Tyr Glu Val Arg Ser Val Ile Asn Val Pro Ser Val
40      275            280            285

```

105

Ile Leu Phe Leu Ser Lys Ser Pro Leu Val Asp Lys Tyr Asp Leu Ser  
 290 295 300  
 Ser Leu Arg Glu Leu Cys Cys Gly Ala Ala Pro Leu Ala Lys Glu Val  
 305 310 315 320  
 5Ala Glu Val Ala Ala Lys Arg Leu Asn Leu Pro Gly Ile Arg Cys Gly  
 325 330 335  
 Phe Gly Leu Thr Glu Ser Thr Ser Ala Ile Ile Gln Thr Leu Gly Asp  
 340 345 350  
 Glu Phe Lys Ser Gly Ser Leu Gly Arg Val Thr Pro Leu Met Ala Ala  
 10 355 360 365  
 Lys Ile Ala Asp Arg Glu Thr Gly Lys Ala Leu Gly Pro Asn Gln Val  
 370 375 380  
 Gly Glu Leu Cys Ile Lys Gly Pro Met Val Ser Lys Gly Tyr Val Asn  
 385 390 395 400  
 15Asn Val Glu Ala Thr Lys Glu Ala Ile Asp Asp Asp Gly Trp Leu His  
 405 410 415  
 Ser Gly Asp Phe Gly Tyr Tyr Asp Glu Asp Glu His Phe Tyr Val Val  
 420 425 430  
 Asp Arg Tyr Lys Glu Leu Ile Lys Tyr Lys Gly Ser Gln Val Ala Pro  
 20 435 440 445  
 Ala Glu Leu Glu Glu Ile Leu Leu Lys Asn Pro Cys Ile Arg Asp Val  
 450 455 460  
 Ala Val Val Gly Ile Pro Asp Leu Glu Ala Gly Glu Leu Pro Ser Ala  
 465 470 475 480  
 25Phe Val Val Lys Gln Pro Gly Thr Glu Ile Thr Ala Lys Glu Val Tyr  
 485 490 495  
 Asp Tyr Leu Ala Glu Arg Val Ser His Thr Lys Tyr Leu Arg Gly Gly  
 500 505 510  
 Val Arg Phe Val Asp Ser Ile Pro Arg Asn Val Thr Gly Lys Ile Thr  
 30 515 520 525  
 Arg Lys Glu Leu Leu Lys Gln Leu Leu Val Lys Ala Gly Gly  
 530 535 540

&lt;210&gt; 224

35&lt;211&gt; 311

&lt;212&gt; PRT

&lt;213&gt; Renilla reniformis

&lt;400&gt; 224

40Met Thr Ser Lys Val Tyr Asp Pro Glu Gln Arg Lys Arg Met Ile Thr

106

|       |     |     |     |
|-------|-----|-----|-----|
| 1     | 5   | 10  | 15  |
| Gly   | Pro | Gln | Trp |
| Trp   | Ala | Arg | Cys |
| Lys   | Gln | Met | Asn |
| Val   | Leu | Asp | Ser |
| 20    | 25  | 30  |     |
| Phe   | Ile | Asn | Tyr |
| Tyr   | Asp | Ser | Glu |
| Lys   | His | Ala | Glu |
| Asn   | Ala | Val | Ile |
| 5     | 35  | 40  | 45  |
| Phe   | Leu | His | Gly |
| Asn   | Ala | Ala | Ser |
| Ser   | Ser | Tyr | Leu |
| Trp   | Arg | His | Val |
| Val   |     |     |     |
| 50    | 55  | 60  |     |
| Pro   | His | Ile | Glu |
| Pro   | Val | Ala | Arg |
| Cys   | Ile | Ile | Pro |
| Asp   | Leu | Ile | Gly |
| 65    | 70  | 75  | 80  |
| 10Met | Gly | Lys | Ser |
| Gly   | Lys | Ser | Gly |
| Asn   | Gly | Ser | Tyr |
| Arg   | Leu | Leu | Asp |
| 85    | 90  | 95  |     |
| His   | Tyr | Lys | Tyr |
| Leu   | Thr | Ala | Trp |
| Phe   | Glu | Leu | Leu |
| Asn   | Leu | Pro | Lys |
| 100   | 105 | 110 |     |
| Lys   | Ile | Ile | Phe |
| Val   | Gly | His | Asp |
| Trp   | Gly | Ala | Cys |
| Leu   | Ala | Phe | His |
| 15    | 115 | 120 | 125 |
| Tyr   | Ser | Tyr | Glu |
| His   | Gln | Asp | Lys |
| Ile   | Lys | Ala | Ile |
| Val   | His | Ala | Glu |
| 130   | 135 | 140 |     |
| Ser   | Val | Val | Asp |
| Val   | Ile | Glu | Ser |
| Trp   | Asp | Glu | Trp |
| Pro   | Asp | Ile | Glu |
| 145   | 150 | 155 | 160 |
| 20Glu | Asp | Ile | Ala |
| Leu   | Ile | Lys | Ser |
| Glu   | Glu | Gly | Glu |
| Lys   | Met | Val | Leu |
| 165   | 170 | 175 |     |
| Glu   | Asn | Asn | Phe |
| Phe   | Val | Glu | Thr |
| Met   | Leu | Pro | Ser |
| Lys   | Ile | Met | Arg |
| 180   | 185 | 190 |     |
| Lys   | Leu | Glu | Pro |
| Glu   | Glu | Phe | Ala |
| Ala   | Tyr | Leu | Glu |
| Pro   | Phe | Lys | Glu |
| 25    | 195 | 200 | 205 |
| Lys   | Gly | Glu | Val |
| Arg   | Arg | Pro | Thr |
| Leu   | Ser | Trp | Pro |
| Arg   | Glu | Ile | Pro |
| 210   | 215 | 220 |     |
| Leu   | Val | Lys | Gly |
| Gly   | Lys | Pro | Asp |
| Val   | Val | Gln | Ile |
| Val   | Arg | Asn | Tyr |
| 225   | 230 | 235 | 240 |
| 30Asn | Ala | Tyr | Leu |
| Arg   | Ala | Ser | Asp |
| Asp   | Leu | Pro | Lys |
| Met   | Phe | Ile | Glu |
| 245   | 250 | 255 |     |
| Ser   | Asp | Pro | Gly |
| Phe   | Phe | Ser | Asn |
| Ala   | Ile | Val | Glu |
| Gly   | Ala | Lys | Lys |
| 260   | 265 | 270 |     |
| Phe   | Pro | Asn | Thr |
| Glu   | Phe | Val | Lys |
| Val   | Lys | Gly | Leu |
| His   | Phe | Ser | Gln |
| 35    | 275 | 280 | 285 |
| Glu   | Asp | Ala | Pro |
| Asp   | Glu | Met | Gly |
| Lys   | Tyr | Ile | Lys |
| Ser   | Phe | Val | Glu |
| 290   | 295 | 300 |     |
| Arg   | Val | Leu | Lys |
| Asn   | Glu | Gln |     |
| 305   | 310 |     |     |



107

&lt;210&gt; 225

&lt;211&gt; 311

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

5

&lt;220&gt;

&lt;223&gt; Sequence of a synthetic luciferase

&lt;400&gt; 225

```

10Met Ala Ser Lys Val Tyr Asp Pro Glu Gln Arg Lys Arg Met Ile Thr
   1             5             10             15
   Gly Pro Gln Trp Trp Ala Arg Cys Lys Gln Met Asn Val Leu Asp Ser
           20             25             30
   Phe Ile Asn Tyr Tyr Asp Ser Glu Lys His Ala Glu Asn Ala Val Ile
15       35             40             45
   Phe Leu His Gly Asn Ala Ala Ser Ser Tyr Leu Trp Arg His Val Val
           50             55             60
   Pro His Ile Glu Pro Val Ala Arg Cys Ile Ile Pro Asp Leu Ile Gly
65       70             75             80
20Met Gly Lys Ser Gly Lys Ser Gly Asn Gly Ser Tyr Arg Leu Leu Asp
           85             90             95
   His Tyr Lys Tyr Leu Thr Ala Trp Phe Glu Leu Leu Asn Leu Pro Lys
           100            105            110
   Lys Ile Ile Phe Val Gly His Asp Trp Gly Ala Cys Leu Ala Phe His
25       115            120            125
   Tyr Ser Tyr Glu His Gln Asp Lys Ile Lys Ala Ile Val His Ala Glu
           130            135            140
   Ser Val Val Asp Val Ile Glu Ser Trp Asp Glu Trp Pro Asp Ile Glu
145       150            155            160
30Glu Asp Ile Ala Leu Ile Lys Ser Glu Glu Gly Glu Lys Met Val Leu
           165            170            175
   Glu Asn Asn Phe Phe Val Glu Thr Met Leu Pro Ser Lys Ile Met Arg
           180            185            190
   Lys Leu Glu Pro Glu Glu Phe Ala Ala Tyr Leu Glu Pro Phe Lys Glu
35       195            200            205
   Lys Gly Glu Val Arg Arg Pro Thr Leu Ser Trp Pro Arg Glu Ile Pro
           210            215            220
   Leu Val Lys Gly Gly Lys Pro Asp Val Val Gln Ile Val Arg Asn Tyr
225       230            235            240
40Asn Ala Tyr Leu Arg Ala Ser Asp Asp Leu Pro Lys Met Phe Ile Glu

```

108

245 250 255  
 Ser Asp Pro Gly Phe Phe Ser Asn Ala Ile Val Glu Gly Ala Lys Lys  
 260 265 270  
 Phe Pro Asn Thr Glu Phe Val Lys Val Lys Gly Leu His Phe Ser Gln  
 5 275 280 285  
 Glu Asp Ala Pro Asp Glu Met Gly Lys Tyr Ile Lys Ser Phe Val Glu  
 290 295 300  
 Arg Val Leu Lys Asn Glu Gln  
 305 310  
 10  
 <210> 226  
 <211> 311  
 <212> PRT  
 <213> Artificial Sequence  
 15  
 <220>  
 <223> Sequence of a synthetic luciferase  
 <400> 226  
 20Met Ala Ser Lys Val Tyr Asp Pro Glu Gln Arg Lys Arg Met Ile Thr  
 1 5 10 15  
 Gly Pro Gln Trp Trp Ala Arg Cys Lys Gln Met Asn Val Leu Asp Ser  
 20 25 30  
 Phe Ile Asn Tyr Tyr Asp Ser Glu Lys His Ala Glu Asn Ala Val Ile  
 25 35 40 45  
 Phe Leu His Gly Asn Ala Ala Ser Ser Tyr Leu Trp Arg His Val Val  
 50 55 60  
 Pro His Ile Glu Pro Val Ala Arg Cys Ile Ile Pro Asp Leu Ile Gly  
 65 70 75 80  
 30Met Gly Lys Ser Gly Lys Ser Gly Asn Gly Ser Tyr Arg Leu Leu Asp  
 85 90 95  
 His Tyr Lys Tyr Leu Thr Ala Trp Phe Glu Leu Leu Asn Leu Pro Lys  
 100 105 110  
 Lys Ile Ile Phe Val Gly His Asp Trp Gly Ala Cys Leu Ala Phe His  
 35 115 120 125  
 Tyr Ser Tyr Glu His Gln Asp Lys Ile Lys Ala Ile Val His Ala Glu  
 130 135 140  
 Ser Val Val Asp Val Ile Glu Ser Trp Asp Glu Trp Pro Asp Ile Glu  
 145 150 155 160  
 40Glu Asp Ile Ala Leu Ile Lys Ser Glu Glu Gly Glu Lys Met Val Leu

109

165 170 175  
 Glu Asn Asn Phe Phe Val Glu Thr Met Leu Pro Ser Lys Ile Met Arg  
 180 185 190  
~~Lys Leu Glu Pro Glu Glu Phe Ala Ala Tyr Leu Glu Pro Phe Lys Glu~~  
 5 195 200 205  
 Lys Gly Glu Val Arg Arg Pro Thr Leu Ser Trp Pro Arg Glu Ile Pro  
 210 215 220  
 Leu Val Lys Gly Gly Lys Pro Asp Val Val Gln Ile Val Arg Asn Tyr  
 225 230 235 240  
 10Asn Ala Tyr Leu Arg Ala Ser Asp Asp Leu Pro Lys Met Phe Ile Glu  
 245 250 255  
 Ser Asp Pro Gly Phe Phe Ser Asn Ala Ile Val Glu Gly Ala Lys Lys  
 260 265 270  
 Phe Pro Asn Thr Glu Phe Val Lys Val Lys Gly Leu His Phe Ser Gln  
 15 275 280 285  
 Glu Asp Ala Pro Asp Glu Met Gly Lys Tyr Ile Lys Ser Phe Val Glu  
 290 295 300  
 Arg Val Leu Lys Asn Glu Gln  
 305 310

20

&lt;210&gt; 227

&lt;211&gt; 311

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

25

&lt;220&gt;

&lt;223&gt; Sequence of a synthetic luciferase

&lt;400&gt; 227

30Met Ala Ser Lys Val Tyr Asp Pro Glu Gln Arg Lys Arg Met Ile Thr  
 1 5 10 15  
 Gly Pro Gln Trp Trp Ala Arg Cys Lys Gln Met Asn Val Leu Asp Ser  
 20 25 30  
 Phe Ile Asn Tyr Tyr Asp Ser Glu Lys His Ala Glu Asn Ala Val Ile  
 35 35 40 45  
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&lt;210&gt; 298

35&lt;211&gt; 542

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

40&lt;223&gt; Sequence of a synthetic luciferase



131

&lt;400&gt; 298

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Met Val Lys Arg Glu Lys Asn Val Ile Tyr Gly Pro Glu Pro Leu His
 1           5           10           15
Pro Leu Glu Asp Leu Thr Ala Gly Glu Met Leu Phe Arg Ala Leu Arg
5           20           25           30
Lys His Ser His Leu Pro Gln Ala Leu Val Asp Val Val Gly Asp Glu
           35           40           45
Ser Leu Ser Tyr Lys Glu Phe Phe Glu Ala Thr Val Leu Leu Ala Gln
           50           55           60
10Ser Leu His Asn Cys Gly Tyr Lys Met Asn Asp Val Val Ser Ile Cys
65           70           75           80
Ala Glu Asn Asn Thr Arg Phe Phe Ile Pro Val Ile Ala Ala Trp Tyr
           85           90           95
Ile Gly Met Ile Val Ala Pro Val Asn Glu Ser Tyr Ile Pro Asp Glu
15           100          105          110
Leu Cys Lys Val Met Gly Ile Ser Lys Pro Gln Ile Val Phe Thr Thr
           115          120          125
Lys Asn Ile Leu Asn Lys Val Leu Glu Val Gln Ser Arg Thr Asn Phe
           130          135          140
20Ile Lys Arg Ile Ile Ile Leu Asp Thr Val Glu Asn Ile His Gly Cys
145          150          155          160
Glu Ser Leu Pro Asn Phe Ile Ser Arg Tyr Ser Asp Gly Asn Ile Ala
           165          170          175
Asn Phe Lys Pro Leu His Phe Asp Pro Val Glu Gln Val Ala Ala Ile
25           180          185          190
Leu Cys Ser Ser Gly Thr Thr Gly Leu Pro Lys Gly Val Met Gln Thr
           195          200          205
His Gln Asn Ile Cys Val Arg Leu Ile His Ala Leu Asp Pro Arg Val
           210          215          220
30Gly Thr Gln Leu Ile Pro Gly Val Thr Val Leu Val Tyr Leu Pro Phe
225          230          235          240
Phe His Ala Phe Gly Phe Ser Ile Thr Leu Gly Tyr Phe Met Val Gly
           245          250          255
Leu Arg Val Ile Met Phe Arg Arg Phe Asp Gln Glu Ala Phe Leu Lys
35           260          265          270
Ala Ile Gln Asp Tyr Glu Val Arg Ser Val Ile Asn Val Pro Ser Val
           275          280          285
Ile Leu Phe Leu Ser Lys Ser Pro Leu Val Asp Lys Tyr Asp Leu Ser
           290          295          300
40Ser Leu Arg Glu Leu Cys Cys Gly Ala Ala Pro Leu Ala Lys Glu Val

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132

305                      310                      315                      320  
 Ala Glu Val Ala Ala Lys Arg Leu Asn Leu Pro Gly Ile Arg Cys Gly  
                          325                      330                      335  
 Phe Gly Leu Thr Glu Ser Thr Ser Ala Asn Ile His Ser Leu Arg Asp  
 5                      340                      345                      350  
 Glu Phe Lys Ser Gly Ser Leu Gly Arg Val Thr Pro Leu Met Ala Ala  
                          355                      360                      365  
 Lys Ile Ala Asp Arg Glu Thr Gly Lys Ala Leu Gly Pro Asn Gln Val  
                          370                      375                      380  
 10Gly Glu Leu Cys Ile Lys Gly Pro Met Val Ser Lys Gly Tyr Val Asn  
                          385                      390                      395                      400  
 Asn Val Glu Ala Thr Lys Glu Ala Ile Asp Asp Asp Gly Trp Leu His  
                          405                      410                      415  
 Ser Gly Asp Phe Gly Tyr Tyr Asp Glu Asp Glu His Phe Tyr Val Val  
 15                      420                      425                      430  
 Asp Arg Tyr Lys Glu Leu Ile Lys Tyr Lys Gly Ser Gln Val Ala Pro  
                          435                      440                      445  
 Ala Glu Leu Glu Glu Ile Leu Leu Lys Asn Pro Cys Ile Arg Asp Val  
                          450                      455                      460  
 20Ala Val Val Gly Ile Pro Asp Leu Glu Ala Gly Glu Leu Pro Ser Ala  
                          465                      470                      475                      480  
 Phe Val Val Lys Gln Pro Gly Lys Glu Ile Thr Ala Lys Glu Val Tyr  
                          485                      490                      495  
 Asp Tyr Leu Ala Glu Arg Val Ser His Thr Lys Tyr Leu Arg Gly Gly  
 25                      500                      505                      510  
 Val Arg Phe Val Asp Ser Ile Pro Arg Asn Val Thr Gly Lys Ile Thr  
                          515                      520                      525  
 Arg Lys Glu Leu Leu Lys Gln Leu Leu Glu Lys Ala Gly Gly  
                          530                      535                      540

30

&lt;210&gt; 299

&lt;211&gt; 1626

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

35

&lt;220&gt;

&lt;223&gt; Sequence of a synthetic luciferase

&lt;400&gt; 299

40atggtgaagc gtgagaaaaa tgtcatctat ggcctgagc ctctccatcc tttggaggat

60

133

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ttgactgccg gcgaaatgct gtttcgtgct ctccgcaagc actctcattt gcctcaagcc 120
ttggtcgatg tggtcggcga tgaatctttg agctacaagg agttttttga ggcaaccgtc 180
ttgctggctc agtccctcca caattgtggc tacaagatga acgacgtcgt tagtatctgt 240
gctgaaaaca ataccggtt cttcattcca gtcacgccc catggtatat cggtatgac 300
5gtggctccag tcaacgagag ctacattccc gacgaactgt gtaaagtcac gggatatctc 360
aagccacaga ttgtcttcac cactaagaat attctgaaca aagtcctgga agtccaaagc 420
cgcaccaact ttattaagcg tatcatcatc ttggacactg tggagaatat tcacggttgc 480
gaatctttgc ctaatttcat ctctcgctat tcagacggca acatcgcaaa ctttaaacca 540
ctccacttcg accctgtgga acaagttgca gccattctgt gtagcagcgg tactactgga 600
10ctoccaaagg gagtcatgca gacccatcaa aacattttgog tgcgtctgat ccatgctctc 660
gatccacgct acggcactca gctgattcct ggtgtcaccg tcttggtcta cttgcctttc 720
ttocatgctt tcggctttca tattactttg gggtacttta tggtcggctc ccgctgatt 780
atgttccgcc gttttgatca ggaggctttc ttgaaagcca tccaagatta tgaagtccgc 840
agtgtcatca acgtgcctag cgtgatcctg tttttgtcta agagccact cgtggacaag 900
15tacgacttgt cttcactgcg tgaatttgtt tgcgggtgcc ctocactggc taaggaggtc 960
gctgaagtgg ccgccaacg cttgaatctt ccagggattc gttgtggctt cggcctcacc 1020
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cgtgtcactc cactcatggc tgctaagatc gctgatcgcg aaactggtaa ggctttgggc 1140
cogaaccaag tgggcgagct gtgtatcaaa ggccctatgg tgagcaaggg ttatgtcaat 1200
20aacgttgaag ctaccaagga ggccatcgac gacgacggct ggttgcatte tggtgatttt 1260
ggatattacg acgaagatga gcattttttac gtcgtggatc gttacaagga gctgatcaaa 1320
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25gaacgtgtga gccatactaa gtacttgcgt ggcggcgtgc gttttgttga ctccatccct 1560
cgtaacgtaa caggcaaaat taccgcaag gagctgttga aacaattgtt ggagaaggcc 1620
ggcgggt 1626

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&lt;210&gt; 300

30&lt;211&gt; 542

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

35&lt;223&gt; Sequence of a synthetic luciferase

&lt;400&gt; 300

Met Val Lys Arg Glu Lys Asn Val Ile Tyr Gly Pro Glu Pro Leu His

1

5

10

15

40Pro Leu Glu Asp Leu Thr Ala Gly Glu Met Leu Phe Arg Ala Leu Arg

134

|  | 20 | 25 | 30 |
|--|----|----|----|
| Lys His Ser His Leu Pro Gln Ala Leu Val Asp Val Val Gly Asp Glu    |    |    |    |
|  | 35 | 40 | 45 |
| Ser Leu Ser Tyr Lys Glu Phe Phe Glu Ala Thr Val Leu Leu Ala Gln    |    |    |    |
| 5 50 55 60   |    |    |    |
| Ser Leu His Asn Cys Gly Tyr Lys Met Asn Asp Val Val Ser Ile Cys    |    |    |    |
| 65 70 75 80  |    |    |    |
| Ala Glu Asn Asn Thr Arg Phe Phe Ile Pro Val Ile Ala Ala Trp Tyr    |    |    |    |
| 85 90 95   |    |    |    |
| 10 Ile Gly Met Ile Val Ala Pro Val Asn Glu Ser Tyr Ile Pro Asp Glu |    |    |    |
| 100 105 110  |    |    |    |
| Leu Cys Lys Val Met Gly Ile Ser Lys Pro Gln Ile Val Phe Thr Thr    |    |    |    |
| 115 120 125  |    |    |    |
| Lys Asn Ile Leu Asn Lys Val Leu Glu Val Gln Ser Arg Thr Asn Phe    |    |    |    |
| 15 130 135 140   |    |    |    |
| Ile Lys Arg Ile Ile Ile Leu Asp Thr Val Glu Asn Ile His Gly Cys    |    |    |    |
| 145 150 155 160  |    |    |    |
| Glu Ser Leu Pro Asn Phe Ile Ser Arg Tyr Ser Asp Gly Asn Ile Ala    |    |    |    |
| 165 170 175  |    |    |    |
| 20 Asn Phe Lys Pro Leu His Phe Asp Pro Val Glu Gln Val Ala Ala Ile |    |    |    |
| 180 185 190  |    |    |    |
| Leu Cys Ser Ser Gly Thr Thr Gly Leu Pro Lys Gly Val Met Gln Thr    |    |    |    |
| 195 200 205  |    |    |    |
| His Gln Asn Ile Cys Val Arg Leu Ile His Ala Leu Asp Pro Arg Tyr    |    |    |    |
| 25 210 215 220   |    |    |    |
| Gly Thr Gln Leu Ile Pro Gly Val Thr Val Leu Val Tyr Leu Pro Phe    |    |    |    |
| 225 230 235 240  |    |    |    |
| Phe His Ala Phe Gly Phe His Ile Thr Leu Gly Tyr Phe Met Val Gly    |    |    |    |
| 245 250 255  |    |    |    |
| 30 Leu Arg Val Ile Met Phe Arg Arg Phe Asp Gln Glu Ala Phe Leu Lys |    |    |    |
| 260 265 270  |    |    |    |
| Ala Ile Gln Asp Tyr Glu Val Arg Ser Val Ile Asn Val Pro Ser Val    |    |    |    |
| 275 280 285  |    |    |    |
| Ile Leu Phe Leu Ser Lys Ser Pro Leu Val Asp Lys Tyr Asp Leu Ser    |    |    |    |
| 35 290 295 300   |    |    |    |
| Ser Leu Arg Glu Leu Cys Cys Gly Ala Ala Pro Leu Ala Lys Glu Val    |    |    |    |
| 305 310 315 320  |    |    |    |
| Ala Glu Val Ala Ala Lys Arg Leu Asn Leu Pro Gly Ile Arg Cys Gly    |    |    |    |
| 325 330 335  |    |    |    |
| 40 Phe Gly Leu Thr Glu Ser Thr Ser Ala Ile Ile Gln Ser Leu Arg Asp |    |    |    |

135

340 345 350  
 Glu Phe Lys Ser Gly Ser Leu Gly Arg Val Thr Pro Leu Met Ala Ala  
 355 360 365  
~~Lys Ile Ala Asp Arg Glu Thr Gly Lys Ala Leu Gly Pro Asn Gln Val~~  
 5 370 375 380  
 Gly Glu Leu Cys Ile Lys Gly Pro Met Val Ser Lys Gly Tyr Val Asn  
 385 390 395 400  
 Asn Val Glu Ala Thr Lys Glu Ala Ile Asp Asp Asp Gly Trp Leu His  
 405 410 415  
 10 Ser Gly Asp Phe Gly Tyr Tyr Asp Glu Asp Glu His Phe Tyr Val Val  
 420 425 430  
 Asp Arg Tyr Lys Glu Leu Ile Lys Tyr Lys Gly Ser Gln Val Ala Pro  
 435 440 445  
 Ala Glu Leu Glu Glu Ile Leu Leu Lys Asn Pro Cys Ile Arg Asp Val  
 15 450 455 460  
 Ala Val Val Gly Ile Pro Asp Leu Glu Ala Gly Glu Leu Pro Ser Ala  
 465 470 475 480  
 Phe Val Val Lys Gln Pro Gly Lys Glu Ile Thr Ala Lys Glu Val Tyr  
 485 490 495  
 20 Asp Tyr Leu Ala Glu Arg Val Ser His Thr Lys Tyr Leu Arg Gly Gly  
 500 505 510  
 Val Arg Phe Val Asp Ser Ile Pro Arg Asn Val Thr Gly Lys Ile Thr  
 515 520 525  
 Arg Lys Glu Leu Leu Lys Gln Leu Leu Glu Lys Ala Gly Gly  
 25 530 535 540

&lt;210&gt; 301

&lt;211&gt; 1626

&lt;212&gt; DNA

30&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Sequence of a synthetic luciferase

35&lt;400&gt; 301

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 ttgactgccg gcgaaatgct gtttcgtgct ctccgcaagc actctcattt gcctcaagcc 120  
 ttggtcgatg tggtcggcga tgaatctttg agctacaagg agttttttga ggcaaccgtc 180  
 ttgctggctc agtccctcca caattgtggc tacaagatga acgacgtcgt tagtatctgt 240  
 40gctgaaaaca ataccggttt cttcattcca gtcatcgccg catggtatat cggatatgatc 300

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gtggctccag tcaacgagag ctacattccc gacgaactgt gtaaagtcac gggatatctct 360
aagccacaga ttgtcttcac cactaagaat attctgaaca aagtcctgga agtccaaagc 420
cgcaccaact ttattaagcg tatcatcatc ttggacactg tggagaatat tcacgggtgc 480
gaatctttgc ctaatttcat ctctcgctat tcagacggca acatcgcaaa ctttaaacca 540
5ctocacttcg accctgtgga acaagttgca gccattctgt gtagcagcgg tactactgga 600
ctcccaaagg gagtcattgca gacccatcaa aacattttgcg tgcgtctgat ccattgctctc 660
gatccacgct acggcactca gctgattcct ggtgtcaccg tcttggtcta cttgcctttc 720
ttccatgctt tcggctttca tattactttg ggttacttta tggtcggtct ccgctgatt 780
atgttcogcc gttttgatca ggaggctttc ttgaaagcca tccaagatta tgaagtcgc 840
10agtgtcatca acgtgcctag cgtgatcctg tttttgtcta agagcccact cgtggacaag 900
tacgacttgt cttactgcg tgaattgtgt tgcgggtgcg ctccactggc taaggaggtc 960
gctgaagtgg ccgccaaacg cttgaatctt ccagggattc gttgtggctt cggcctcacc 1020
gaatctacca gtgcgattat ccagactctc ggggatgagt ttaagagcgg ctctttgggc 1080
cgtgtcactc cactcatggc tgctaagatc gctgatcgcg aaactggtaa ggctttgggc 1140
15ccgaaccaag tgggcgagct gtgtatcaaa ggccctatgg tgagcaaggg ttatgtcaat 1200
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gaacgtgtga gccatactaa gtacttgctg ggcggcgtgc gttttgttga ctccatccct 1560
cgtaacgtaa caggcaaaat taccgcgaag gagctgttga aacaattgtt ggtgaaggcc 1620
ggcggt 1626

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25<210> 302

<211> 542

<212> PRT

<213> Artificial Sequence

30<220>

<223> Sequence of a synthetic luciferase

<400> 302

Met Val Lys Arg Glu Lys Asn Val Ile Tyr Gly Pro Glu Pro Leu His

35 1 5 10 15

Pro Leu Glu Asp Leu Thr Ala Gly Glu Met Leu Phe Arg Ala Leu Arg

20 25 30

Lys His Ser His Leu Pro Gln Ala Leu Val Asp Val Val Gly Asp Glu

35 40 45

40Ser Leu Ser Tyr Lys Glu Phe Phe Glu Ala Thr Val Leu Leu Ala Gln

137

|  |     |     |
|--|-----|-----|
| 50   | 55  | 60  |
| Ser Leu His Asn Cys Gly Tyr Lys Met Asn Asp Val Val Ser Ile Cys    |     |     |
| 65   | 70  | 75  |
| Ala Glu Asn Asn Thr Arg Phe Phe Ile Pro Val Ile Ala Ala Trp Tyr    |     |     |
| 5  | 85  | 90  |
| Ile Gly Met Ile Val Ala Pro Val Asn Glu Ser Tyr Ile Pro Asp Glu    |     |     |
|  | 100 | 105 |
| Leu Cys Lys Val Met Gly Ile Ser Lys Pro Gln Ile Val Phe Thr Thr    |     |     |
|  | 115 | 120 |
| 10 Lys Asn Ile Leu Asn Lys Val Leu Glu Val Gln Ser Arg Thr Asn Phe |     |     |
|  | 130 | 135 |
| Ile Lys Arg Ile Ile Ile Leu Asp Thr Val Glu Asn Ile His Gly Cys    |     |     |
| 145  | 150 | 155 |
| Glu Ser Leu Pro Asn Phe Ile Ser Arg Tyr Ser Asp Gly Asn Ile Ala    |     |     |
| 15   | 165 | 170 |
| Asn Phe Lys Pro Leu His Phe Asp Pro Val Glu Gln Val Ala Ala Ile    |     |     |
|  | 180 | 185 |
| Leu Cys Ser Ser Gly Thr Thr Gly Leu Pro Lys Gly Val Met Gln Thr    |     |     |
|  | 195 | 200 |
| 20 His Gln Asn Ile Cys Val Arg Leu Ile His Ala Leu Asp Pro Arg Tyr |     |     |
|  | 210 | 215 |
| Gly Thr Gln Leu Ile Pro Gly Val Thr Val Leu Val Tyr Leu Pro Phe    |     |     |
| 225  | 230 | 235 |
| Phe His Ala Phe Gly Phe His Ile Thr Leu Gly Tyr Phe Met Val Gly    |     |     |
| 25   | 245 | 250 |
| Leu Arg Val Ile Met Phe Arg Arg Phe Asp Gln Glu Ala Phe Leu Lys    |     |     |
|  | 260 | 265 |
| Ala Ile Gln Asp Tyr Glu Val Arg Ser Val Ile Asn Val Pro Ser Val    |     |     |
|  | 275 | 280 |
| 30 Ile Leu Phe Leu Ser Lys Ser Pro Leu Val Asp Lys Tyr Asp Leu Ser |     |     |
|  | 290 | 295 |
| Ser Leu Arg Glu Leu Cys Cys Gly Ala Ala Pro Leu Ala Lys Glu Val    |     |     |
| 305  | 310 | 315 |
| Ala Glu Val Ala Ala Lys Arg Leu Asn Leu Pro Gly Ile Arg Cys Gly    |     |     |
| 35   | 325 | 330 |
| Phe Gly Leu Thr Glu Ser Thr Ser Ala Ile Ile Gln Thr Leu Gly Asp    |     |     |
|  | 340 | 345 |
| Glu Phe Lys Ser Gly Ser Leu Gly Arg Val Thr Pro Leu Met Ala Ala    |     |     |
|  | 355 | 360 |
| 40 Lys Ile Ala Asp Arg Glu Thr Gly Lys Ala Leu Gly Pro Asn Gln Val |     |     |

138

|   |     |     |
|---|-----|-----|
| 370   | 375 | 380 |
| Gly Glu Leu Cys Ile Lys Gly Pro Met Val Ser Lys Gly Tyr Val Asn   |     |     |
| 385   | 390 | 395 |
| Asn Val Glu Ala Thr Lys Glu Ala Ile Asp Asp Asp Gly Trp Leu His   |     | 400 |
| 5   | 405 | 410 |
| Ser Gly Asp Phe Gly Tyr Tyr Asp Glu Asp Glu His Phe Tyr Val Val   |     | 415 |
|   | 420 | 425 |
| Asp Arg Tyr Lys Glu Leu Ile Lys Tyr Lys Gly Ser Gln Val Ala Pro   |     | 430 |
|   | 435 | 440 |
| 10Ala Glu Leu Glu Glu Ile Leu Leu Lys Asn Pro Cys Ile Arg Asp Val |     | 445 |
|   | 450 | 455 |
| Ala Val Val Gly Ile Pro Asp Leu Glu Ala Gly Glu Leu Pro Ser Ala   |     | 460 |
|   | 465 | 470 |
| Phe Val Val Lys Gln Pro Gly Thr Glu Ile Thr Ala Lys Glu Val Tyr   |     | 475 |
| 15  | 485 | 490 |
| Asp Tyr Leu Ala Glu Arg Val Ser His Thr Lys Tyr Leu Arg Gly Gly   |     | 495 |
|   | 500 | 505 |
| Val Arg Phe Val Asp Ser Ile Pro Arg Asn Val Thr Gly Lys Ile Thr   |     | 510 |
|   | 515 | 520 |
| 20Arg Lys Glu Leu Leu Lys Gln Leu Leu Val Lys Ala Gly Gly         |     | 525 |
|   | 530 | 535 |
|   |     | 540 |